Society of Toxicology

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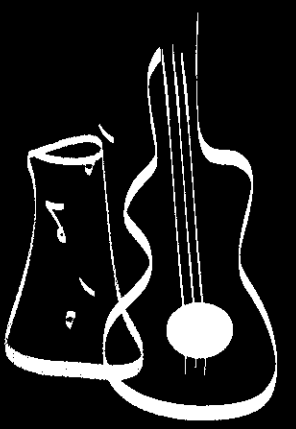

An Official Journal of the Society of Toxicology
Supplement

TOXICOLOGICAL SCIENCES

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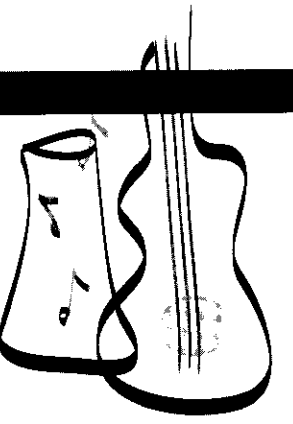

An Official Journal of the Society of Toxicology
Supplement

TOXICOLOGICAL SCIENCES
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Abstracts of the 41st Annual Meeting

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, roundtable, and poster sessions of the 41st Annual Meeting of the Society of Toxicology, held at the Opryland Hotel and Convention Center, Nashville, Tennessee, March 17–21, 2002.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 385.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 411.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

Additional Late-Breaking Abstracts are issued in a supplement to this publication and are available at the 41st Annual Meeting and through the Society of Toxicology Headquarters office.

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1 USE OF LASER CAPTURE MICRODISSECTION (LCM) IN MOLECULAR TOXICOLOGY RESEARCH.

J. I. Everitt. *CIIT Centers for Health Research, Research Triangle Park, NC.*

Laser capture microdissection (LCM) is a recently developed technique that allows one to rapidly procure morphologically defined cell populations from sections of heterogenous tissues using direct visualization. This technique has greatly expanded the ability of the toxicologist to conduct molecular analyses on a wide array of specific target cells and tissues of interest. Cells obtained by microdissection have been used as a source of genomic DNA, the isolation of mRNA amenable to reverse transcription polymerase chain reaction (RT-PCR), and the generation of expression libraries. LCM has been combined with cDNA microarray hybridization techniques and proteomic methods to provide new and exciting approaches for combining gene expression with traditional morphological methods. This seminar will review microdissection methods and equipment and discuss the utility of LCM in toxicology studies.

2 A PRACTICAL APPROACH TO BLOOD AND LYMPHOID TISSUE (BLT) IN TOXICOLOGY ASSESSMENTS.

J. C. Schuh[1], L. Lanning[2], G. Travlos[3], P. Mann[4] and M. Leach[5]. *[1]Applied Veterinary Pathobiology, Bainbridge Island, WA, [2]BioReliance, Rockville, MD, [3]NIEHS, Research Triangle Park, NC, [4]Experimental Pathology Labs, Inc, Research Triangle Park, NC and [5]BASF, Worcester, MA.*

Blood, bone marrow, thymus, spleen, lymph nodes and mucosa-associated lymphoid tissue are a complicated but important interactive system of tissues and cells modulated directly and indirectly by xenobiotics. Evaluation of blood and lymphoid tissues (BLT) has always been part of a standard histopathology and toxicology screen but advanced evaluations of BLT are becoming increasingly important to the rapidly changing fields of immunotoxicology and immunotherapeutic development. The objective of this basic course is to provide contemporary information on the pathophysiology of BLT useful to individuals in regulatory and research areas of toxicology. This course will review important features of i) basic anatomy, function, and evaluation of blood and blood forming organs, ii) anatomy and function of lymphoid tissues and their component parts, iii) terminology, iv) general and toxic immunomodulation, and v) pathophysiology (neoplastic and non-neoplastic responses). Species, sex and age specific differences that may affect the design and outcome of studies, and techniques used to evaluate BLT will be discussed. A practical understanding of the anatomy, terminology, and toxicologic pathology associated with BLT, will aid toxicologists in making proper interpretation of treatment-related changes in safety and efficacy studies, and in communicating this information between disciplines, within teams and to regulatory agencies.

3 ALTERATIONS IN GENE EXPRESSION AS A MECHANISM OF TOXICANT ACTION.

R. N. Hines[1], Q. Ma[2], G. K. Andrews[3] and P. E. Mirkes[4]. *[1]Medical College of Wisconsin, Milwaukee, WI, [2]CDC/NIOSH, Morgantown, WV, [3]University of Kansas Medical Center, Kansas City, KS and [4]University of Washington, Seattle, WA.*

Over the last several years, it has become apparent that many environmental toxicants exert their effects by the activation or disruption of specific signaling pathways, ultimately resulting in alterations in gene expression. With the completion of the human genome project and the advent of many powerful new technologies, there has been a revolution in our understanding of these mechanisms on the molecular level. The proposed continuing education course is designed to review our current state of knowledge regarding toxicant-induced alterations in gene expression and also identify future directions and research opportunities. The first speaker will focus on our current understanding of the mechanism(s) whereby four receptors, i.e., the Ah receptor (AhR), the Constitutive Androstane Receptor (CAR), the Pregnane X Receptor (PXR), and the Peroxisome Proliferator Activated Receptor (PPAR), mediate the toxicity of four broad classes of chemicals. In contrast to these specific receptor mechanisms, metals exert their toxicity through both stress-response pathways, as well as specific metal-responsive transcription factors. The second speaker will focus on our current understanding of these pathways of toxicant action. The third speaker will review the many exciting discoveries in our understanding of how toxicants alter gene expression during specific windows of development and thereby exert their teratogenic effects. Finally, the fourth speaker will discuss the role of tissue-selective transcription factors on the expression of xenobiotic metabolizing enzymes and how this process impacts toxicant susceptibility. As an advanced course, this curriculum should appeal to toxicologists whose research is in or immediately peripheral to this focus area, but who are interested in gaining a better understanding of the overall subject and its future direction.

4 INTEGRATING TOXICOLOGIC PATHOLOGY INTO COMPOUND EVALUATION AND RISK ASSESSMENT.

D. C. Wolf[1], J. F. Hardisty[2], R. T. Miller[3] and J. I. Everitt[4]. *[1]USEPA, Durham, NC, [2]Experimental Pathology Laboratory, Research Triangle Park, NC, [3]GlaxoSmithKline, Research Triangle Park, NC and [4]CIIT Centers for Health Research, Research Triangle Park, NC.*

Pathology endpoints are the central response around which human health risk assessment is determined. This course is designed for the general toxicology community to gain an understanding of the basics of toxicologic pathology. Toxicologic pathology encompasses the study of changes in tissue morphology that help define the risk of exposure to xenobiotics. The first presentation will review the basics of pathology studies including tissue processing, pathology review, standard techniques, and efforts being made to standardize the conduct, review, and reporting of pathology studies which are important for appropriate interpretation of data. Other speakers will discuss the structural and functional aspects of the liver and kidney and the general concepts of mechanisms of injury, species and sex differences, background and induced lesions, which are necessary for appropriate risk characterization. The liver is the most common target for xenobiotic-induced adverse effects and the kidney has a central role in filtration, metabolism, and excretion and is frequently a site of toxic injury. Correlating clinicopathology with morphologic and functional alterations is necessary for full understanding of adverse effects. Finally, diagnostic terminology, study data relative to cancer bioassay findings, the steps in tumor development, and their relevance to human health risk will be presented.

5 BASIC PRINCIPLES AND PROTOCOLS IN MOLECULAR TOXICOLOGY.

W. B. Mattes[1], J. W. Davis, II[2], M. K. Walker[3], J. P. Vanden Heuvel[4], M. S. Denison[5] and C. B. Marcus[3]. *[1]Pharmacia, Kalamazoo, MI, [2]Schering Plough Institute, Lafayette, NJ, [3]University of New Mexico, Albuquerque, NM, [4]Pennsylvania State University, University Park, PA and [5]University of California- Davis, Davis, CA.*

Many of the mechanisms through which xenobiotics affect tissues or cells occur at the molecular level. Over the past ten or fifteen years the use of molecular techniques to dissect mechanisms of toxicity has grown greatly. These techniques are used to identify growth regulatory pathways, alterations in gene and/or protein expression, as well as protein:DNA and protein:protein interactions. Accordingly, there has been an explosion in the number of reagents and kits that are commercially available. While these kits and reagents have facilitated the detection of mechanisms of toxicity, a basic understanding of the methods used is just as important. This course will detail a number of basic techniques currently in use in an attempt to give a researcher new to this area information as to which tools may be most relevant with regards to their specific research area. Presentations will include the practical considerations when setting up a given technique as well as references that will help the investigator trouble shoot these systems. Finally, actual data will be shown in an effort to demonstrate the kinds of information that can be obtained by these experiments and the ways in which this information can be interpreted and used to develop hypothesis-driven research. This is a basic level course intended to introduce to the researcher the tools and references that are available to him or her.

6 TWO-STEPPING THROUGH TOXICOGENOMICS: A BASIC PRIMER.

M. J. Cunningham[1], T. Zacharewski[2], R. Somogyi[1] and B. A. Merrick[3]. *[1]Molecular Mining Corporation, Kingston, ON, Canada, [2]Michigan State University, East Lansing, MI and [3]NIEHS, Research Triangle Park, NC.*

Toxicogenomics as used here is broadly defined as gene and protein expression technologies and their application to addressing pertinent issues of toxicology. This basic course will start with an overview of how genomics and proteomics came into existence. Different microarray formats will be covered including cDNA, oligonucleotide, fiber optic, and high-throughput versions of gene expression microarrays as well as a broad overview on proteomics and data analysis. Several landmark papers will be discussed showing how genomics and proteomics can be applied. An in-depth presentation will follow detailing how to set up and run your own microarrays and will cover array manufacture, sample preparation, array hybridization and scanning, and image analysis. In addition, setting up and running 2D protein gels will be discussed as well as their interpretation using mass spectroscopy. With all of these technologies, complex data sets are generated and the final presentation will discuss alternative statistical and bioinformatic methods which can be used to analyze the data.

7 CHALLENGES IN DEVELOPMENT OF ANTICANCER DRUGS.

V. Reddy[1], A. Smith[2], J. T. Tomaszeski[2], R. E. Parchment[3], J. K. Leighton[4], J. Wright[2] and A. Worobec[4]. *[1]Eli Lilly and Company, Greenfield, IN, [2]National Cancer Institute, Rockville, MD, [3]Karmanos Cancer Institute, Detroit, MI and [4]USFDA, Rockville, MD.*

Preclinical and clinical drug development for anticancer drugs differ from other pharmaceuticals, because of the life-threatening nature of the disease. Treatment with anticancer drugs at clinically efficacious doses usually induces serious side effects but often less threatening to patients than their disease. The design of preclinical toxicology studies for anticancer drugs is intended to identify a safe clinical starting dose, characterize toxicities that may be encountered in human clinical trial, and determine whether these toxicities are reversible, manageable, and predictable. This basic course will focus on different aspects of preclinical and clinical anticancer drug development. The first speaker will present differences in the preclinical drug development philosophy between different classes of anticancer drugs (e.g., cytotoxic, immunomodulators, and modulatory drugs). The second speaker will discuss the prediction of human tolerated dose using *in vitro* hematotoxicity tests and the integration of this knowledge into preclinical modeling and toxicology. The third speaker will focus on regulatory considerations for preclinical development of anticancer drugs. The fourth speaker will discuss the clinical development of anticancer drugs. The final speaker will review the special preclinical and clinical regulatory issues associated with the development of biologics for the treatment of cancer. This basic drug development course is targeted to government, biotechnology and pharmaceutical toxicologists, as well as physicians and general toxicologists with an interest in cancer chemotherapy.

8 INCORPORATION OF PHARMACOKINETIC AND PHARMACODYNAMIC DATA INTO RISK ASSESSMENTS.

J. C. Lipscomb[1], L. Haber[2], B. Meek[3], K. Krishnan[4], G. L. Kedderis[5] and H. Clewell[6]. *[1]USEPA / ORD/NCEA, Cincinnati, OH, [2]Toxicology Excellence for Risk Assessment, Cincinnati, OH, [3]Health Canada, Ottawa, ON, Canada, [4]University of Montreal, Montreal, PQ, Canada, [5]CIIT Centers for Health Research, Research Triangle Park, NC and [6]ENVIRON Corporation, Ruston, LA.*

The increasing attention to chemical-specific mechanistic data in human health risk assessments should encourage researchers and programmers to identify and develop risk-relevant information. Recently, guidance for replacing default uncertainty factors with adjustment factors based on chemical-specific data has been made available. This course will investigate recent and emerging approaches to metabolism, pharmacokinetics, and pharmacodynamics with respect to producing data adequate for inclusion in human health risk assessments, and methods to use such data in risk assessment. Physiologically-based pharmacokinetic (PBPK) modeling has developed risk-relevant information, but the "validation" of these models for some chemicals in humans may be problematic. In some instances, the best available data may be generated *in vitro* and *in silico* and require extrapolation to the *in vivo* setting. PBPK models then become attractive and can be used to estimate risk-relevant, mechanistically-related PK outcomes, and variance thereof, when adequate biochemical and physiologic/anatomic information are incorporated. Human interindividual variance is presently addressed in the uncertainty factors (UFH) used to derive safe levels of exposure. The quantification of human variance through *in vivo, in vitro,* and *in silico* approaches will be presented. This will include the evaluation of genetic and environmental modulation of biochemical individuality (e.g., polymorphisms and co-exposures) and their impact on tissue dosimetry and age-related differences in humans.

9 TOXICOLOGY OF NATURALLY OCCURRING TOXINS - DON'T MESS WITH MOTHER NATURE!

F. W. Oehme[1], R. W. Coppock[2], K. L. Wallace[3], M. E. Peterson[4], B. Furbee[6] and R. Ko[5]. *[1]Kansas State University, Manhattan, KS, [2]Alberta Research Council, Vegreville, AB, Canada, [3]Good Samaritan Regional Medical Care-Phoenix, Phoenix, AZ, [4]Reid Veterinary Hospital, Albany, OR, [5]California Deparatment of Health Seravices, Sacramento, CA and [6]Indiana Poison Center, Indianapolis, IN.*

Natural toxins are generally not recognized for their true importance. The benign perception that nature is all healing is contradicted by the numerous but often little appreciated public risks from contact with venomous animals and use of plants as herbal remedies. Lack of understanding these hazards produces a false sense of societal security. To update the SOT membership about the range, potency, and mechanisms of action of poisons found in nature, this basic course will illustrate the chemical risks, compositions, mechanisms of action, effects and effective therapies for animal venoms, growing plants, and herbal products. Each speaker is a nationally recognized expert in their presentation's themes. Drs. Wallace and Peterson recognize and manage snake, reptile, and arthropod envenomations in their daily toxicology practices. Poison Center director, Dr. Furbee, overviews repeated exposures to poisonous plants with a working knowledge of each plant's poisonous principle(s). Dr. Ko's west coast laboratory deals with the variety of toxicology events from herbal products. Each speaker will provide personal experiences and the characteristics, toxicologic mechanisms and effects of specific intoxications, alerting toxicologists more familiar with sterile and urban environments with the circumstances and dangers afforded by Mother Nature. This unique continuing education course gives insight into the living dangers around us by providing current toxicological knowledge about these everyday lifestyle hazards. "Naturally Occurring Toxins" will be timely, of wide professional and public interest, and certainly stimulating to our broad audience.

10 REGULATION OF DRUG AND CHEMICALLY-INDUCED APOPTOTIC CELL DEATH: NEW *IN VIVO* PERSPECTIVES.

S. D. Ray[1], S. B. Pruett[2], J. Kang[3] and N. Jena[4]. *[1]Long Island University, Brooklyn, VA, [2]Louisiana State University Medical Center, Shreveport, LA, [3]University of Louisville, Louisville, KY and [4]M.I.T., Cambridge, MA.*

Apoptosis is a natural consequence *in vivo,* and there is now substantial evidence that apoptosis plays an important role in the toxic effects of a number of drugs and chemicals. Although it is a naturally-orchestrated self-limiting program, a vast majority of the investigators employ *in vitro* models to interpret *in vivo* mechanisms. While *in vitro* assays are relatively sensitive, specific, and reliable, an ongoing question is the reproducibility of such mechanisms in complex *in vivo* systems. Therefore, one of the primary goals of this course is to discuss coherently numerous proposed pathways that regulate this cell suicidal process and test their feasibility in *in vivo* models. The overall objectives of this CE course are: (i) an overview of role of apoptosis during target organ toxicities (cardiotoxicity, hepatotoxicity, nephrotoxicity, and immunotoxicity), (ii) a broad review of mechanisms of action of target-organ specific apoptogenic drugs and chemicals; and (iii) a discussion of mechanisms that regulate apoptosis at the organ, cellular, sub cellular, and molecular levels. Since oxidative stress, caspases, caspase-activated DNAse, reactive oxygen species, mitochondrial, and cell cycle-related events are known to modulate this process, their contributory roles will be a brief part of the curriculum. The concepts gained from this course will be useful to teachers and researchers involved in target organ toxicology, biochemical toxicology, general toxicology, carcinogenesis, molecular toxicology and mechanistic toxicology.

11 INTERNAL DOSIMETRY: MEASUREMENT OF DNA DAMAGE AS AN INDICATOR OF INTERNAL EXPOSURE TO GENOTOXICANTS.

B. S. Shane[1], M. Poirier[2], L. Marnett[3], R. Tice[4] and B. Finette[5]. *[1]Integated Laboratory Systems, Research Triangle Park, NC, [2]NIH, Bethesda, MD, [3]Vanderbilt University Medical Center, Nashville, TN, [4]Integrated Laboratory Systems, Durham, NC and [5]University of Vermont, Burlington, VT.*

Large molecular weight chemical carcinogens, including polycyclic aromatic hydrocarbons, aromatic and heterocyclic amines, and aflatoxins, are encountered in the ambient environment, the work place and in our food supply. These carcinogens usually require metabolic activation before becoming adducted to DNA. The covalent DNA adducts which are formed, often termed "bulky" adducts, distort the conformation of the DNA helix and are typically removed from the DNA by nucleotide excision repair. However, some proportion of these adducts are not repaired and following cell division can result in a mutation. Other pro-mutagenic events (malondialdehyde-DNA adducts) occur as the result of oxidative damage resulting from endogenous metabolic processes and exposure to xenobiotics that initiate a lipid peroxidation cascade. A third type of DNA damage results from agents that induce strand breaks or crosslinking. The first two talks will discuss the spectrometric-, immunochemical-, and postlabeling-based approaches that can be used to measure both bulky adducts and those caused by lipid peroxidation products. The third talk will describe the single cell gel (Comet) assay that is used to measure strand breaks, alkali-labile sites, and crosslinking. The final talk will focus on the consequence of DNA damage, namely mutations, particularly those found in infants and young children. The measurement of these mutations using the T-lymphocyte cloning assay that quantifies the mutant frequency (MF) of the HPRT gene will be described. This course will be suitable for those toxicologists that are interested in the latest techniques used to measure DNA damage and how these endpoints can be used as an internal dosimeter for epidemiological monitoring of human populations.

12 TOXICITY PROFILING OF GENES AND PROTEINS BY TOXICOLOGISTS: ADVANCED TOPICS IN TOXICOGENOMICS.

B. A. Merrick[1], D. Dixon[1], C. Corton[2], P. Bushel[1], F. A. Witzmann[3] and S. Rabatin[4]. [1]*NIEHS, Research Triangle Park, VA,* [2]*CIIT Centers for Health Research, Research Triangle Park, NC,* [3]*Indiana University Medical School, Indianapolis, IN and* [4]*Applied Biosystems, Santa Fe, NM.*

Chemical toxicity profiling using gene expression technologies will soon become more accessible to practicing toxicologists through contract, industrial, and academic core facilities. It is anticipated that gene expression data for many compounds will begin to appear in preclinical drug evaluations, new drug applications, and environmental risk assessment documents as well as basic toxicology research. Although many toxicologists have an understanding of genomics and proteomics, the best use of their applications, attendant bioinformatics, and associated databases still present a challenge. This advanced course is aimed at research and industrial toxicologists to better familiarize them with gene expression technologies at the transcript and protein level. The course is structured around four areas, genomics, genoinformatics, proteomics, and proteoinformatics. Experts in each area will cover the latest technological advances, specific applications and how to relate and interpret the high toxicogenomic information density with bioinformatic tools and relevant databases.

13 STRATEGIES AND ISSUES IN NON-CLINICAL DEVELOPMENT OF INTRAVENOUS INFUSION DRUG PRODUCTS.

W. Halliwell[1], G. Lulham[2], G. Washer[3], K. Hew[4] and J. A. Cavagnaro[5]. [1]*Schering-Plough Research Institute, Lafayette, NJ,* [2]*JTI MacDonald Corp., Toronto, ON, Canada,* [3]*ClinTrials BioResearch, Ltd., Senneville (Montreal), PQ, Canada,* [4]*Purdue Pharma L.P., Ardsley, NY and* [5]*Access BIO, Leesburg, VA.*

A large number of pharmaceutical and biotechnology products intended for administration by intravenous infusion in the clinic have entered into non-clinical development during the past decade. Many of these have shown early success in the clinic and subsequently required additional safety evaluation. Non-clinical testing has also utilized the intravenous route to avoid issues associated with short drug half-life or poor absorption that preclude using more traditional clinical dosing routes. Additionally, continuous infusion studies are conducted with non-infusion drugs to control blood levels, such as fetal/neonatal exposure, for mechanistic studies. As a result, the use of intravenous infusion for non-clinical studies has increased steadily over the past number of years. There are unique design, conduct, and interpretation issues associated with this route of drug administration as a tool in evaluating product safety. Toxicologists and regulators in pharmaceutical/biotech product development are increasingly likely to be involved in designing, conducting, interpreting, and assessing mechanisms of toxicity for non-clinical safety programs using the intravenous infusion route. The goal of this course is to explore the scientific and technical challenges associated with the use of continuous and intermittent intravenous infusion in drug development. The utility and special considerations of this treatment route for reproductive studies of intravenously infused drug products and non-infusion products where there may be a need to achieve specific systemic exposures will also be addressed.

14 CURRENT APPROACHES FOR VALIDATION AND REGULATORY ACCEPTANCE OF NOVEL TEST METHODS.

S. Green[1], L. M. Schechtman[3], W. S. Stokes[2], K. Hamernik[4], A. Worth[5] and H. Koeter[6]. [1]*Howard University College of Medicine, Washington, DC,* [2]*NIEHS, Research Triangle Park, NC,* [3]*USFDA, Rockville, MD,* [4]*USEPA, Washington, DC,* [5]*ECVAM, Varese, Italy and* [6]*OECD, Paris, France.*

New technologies such as toxicogenomics are providing a mechanistic basis for the improvement of existing toxicity testing methods and for the development of new improved methods. Such methods may be faster, more economical, and reduce animal use. In order for regulatory authorities and industries to embrace methods using new technologies, there must be an effective process for determining their scientific validity and acceptability for regulatory use. Enactment of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Authorization Act of 2000 established ICCVAM as a permanent committee, and requires federal agencies to determine that methods are valid for their proposed use prior to their adoption. Federal regulatory agencies have implemented processes to consider the applicability and acceptability of ICCVAM-recommended methods, and to inform the regulated community of their decisions. A parallel organization has been established in the European Union (European Center for the Validation of Alternative Methods, ECVAM). Five methods have successfully completed the ICCVAM and ECVAM evaluation/validation processes and have been adopted by regulatory authorities. International acceptance of new methods is an essential prerequisite for widespread use. This course will review new initiatives by national and international authorities to achieve acceptance of new and revised toxicological testing methodologies. Current issues relevant to validation and regulatory acceptance will be addressed. This course is targeted for toxicologists and scientists involved in developing, validating, or using toxicological test methods to meet national and international regulatory testing requirements. The course is particularly relevant as differential gene expression is rapidly being applied to new and existing test methods to identify more sensitive and specific decision criteria.

15 IMAGING THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE IN TOXIC INJURY.

D. A. Fox[1] and J. J. Lemasters[2]. [1]*University of Houston, Houston, TX and* [2]*University of North Carolina, Chapel Hill, NC.*

Mitochondria in excitable and non-excitable tissues are the direct and indirect targets of many xenobiotics that produce apoptotic and necrotic cell death. Determination of the temporal and sequential relationships between the opening of the mitochondrial permeability transition (PT) pore, mitochondrial depolarization and swelling, cytochrome c release and caspase activation during cell death and of the molecular mechanisms underlying each of these events are critically important. In toxicology, understanding the role and molecular mechanisms of PT pore opening will allow the development of pharmacological and genetic strategies to prevent inappropriate apoptosis as well as to initiate and control the apoptotic process for therapeutic purposes. Current evidence suggests that the PT pore is a complex of the voltage-dependent anion channel (VDAC), the adenine nucleotide translocase (ANT) and cyclophilin-D (CyP-D) formed at contact sites between the inner and outer mitochondrial membranes. This symposium provides four presentations from investigators using state-of-the-art imaging techniques such as confocal microscopy and electron tomography as well as biochemical and cell biological techniques to determine the mechanisms of PT opening and the role of these proteins in the PT pore during exposure to ethanol, heavy metals, solvents and under conditions that produce hypoxia, calcium overload and death receptor ligation. The presentations will explore intrinsic cellular and tissue differences in susceptibility to PT pore opening as well as outcomes in heart, liver, brain and retina in developing and adult tissues and isolated cells. Detailed mechanistic studies also will provide metabolic, pharmacological and transgenic strategies for cytoprotection. The major objective of this symposium is to present and discuss up-to-date knowledge on the role of PT pores in toxicology using techniques at the single cell level. This symposium should have broad appeal to all toxicologists examining mechanisms of cell death.

16 PATHOPHYSIOLOGY OF THE MITOCHONDRIAL PERMEABILITY TRANSITION.

P. Bernardi[1], V. Petronilli[1] and F. Di Lisa[2]. [1]*Biomedical Sciences, University of Padova, Padova, Italy and* [2]*Biological Chemistry, University of Padova, Padova, Italy.*
Sponsor: D. Fox.

We are studying the properties of the mitochondrial permeability transition pore (PTP) in cells and tissues, and we describe here our results on the relationships between PTP opening and cell death in two models. (i) We have investigated the relationship between PTP opening, mitochondrial depolarization, cytochrome c release and occurrence of cell death in MH1C1 hepatoma cells treated with a Ca^{2+} ionophore or arachidonic acid, a potent PTP inducer. We could detect both transient and long lasting PTP openings. While cell viability was hardly affected by PTP openings of short duration, longer PTP openings caused mitochondrial depolarization followed by release of cytochrome c and cell death. Thus, modulation of PTP open time appears to be the key element in determining the outcome of stimuli that converge on the PTP. (ii) We have studied the role of the PTP in ischemia-reperfusion damage of the heart by investigating metabolism of NAD^+, which is released from mitochondria when the PTP opens and then hydrolyzed by glycohydrolase, an enzyme associated with the outer mitochondrial membrane. Perfused rat hearts subjected to global ischemia for 30 min displayed a 30% decrease in tissue NAD+ content, which was not modified by extending the duration of ischemia. Reperfusion resulted in a more severe reduction of both total and mitochondrial contents of NAD^+, which could be measured in the coronary effluent together with lactate dehydrogenase. The addition of 0.2 μM cyclosporin A (CsA) or of its analogue MeVal-4-Cs maintained higher NAD^+ contents, especially in mitochondria, and protected the heart from reperfusion damage. Thus, PTP opening in the heart can be documented as a CsA-sensitive release of NAD^+, which is then partly degraded by glycohydrolase and partly released when sarcolemmal integrity is compromised. Once released from the matrix, NAD^+ could be transformed into cyclic ADPribose, thus promoting Ca^{2+} release from intracellular stores and amplifying the effects exerted by an initial rise of $[Ca^{2+}]_i$.

17 THREE-DIMENSIONAL RECONSTRUCTIONS OF ROD MITOCHONDRIA AFTER LEAD-INDUCED APOPTOSIS REVEAL AN INCREASE IN CONTACT SITES THAT IS BLOCKED BY Bcl-xL OVEREXPRESSION.

G. A. Perkins[1], L. He[2], A. T. Poblenz[3] and D. A. Fox[4]. *[1]Neurosciences, Univ. California, San Diego, La Jolla, CA, [2]Cell Biology, Univ. North Carolina, Chapel Hill, NC, [3]MD Anderson, Univ. Texas, Houston, TX and [4]College of Optometry, Univ. Houston, Houston, TX.*

Developmental lead exposure in rats and mice produces a progressive loss of rods that correlates with an apoptotic mechanism driven by retinal lead and calcium overload. We used wild-type and transgenic mice overexpressing Bcl-xL only in photoreceptors to examine the influence of Bcl-xL on mitochondria in lead-induced apoptosis. Our previous *in vitro* data demonstrated that Bcl-xL exerts its cytoprotective effect at the mitochondrial level. We accumulated evidence that the mitochondrial permeability transition (MPT) is the apoptotic mechanism in wild-type mice exposed to lead and is blocked by Bcl-xL overexpression. This is the first *in vivo* evidence that Bcl-xL overexpression regulates apoptosis. Because the MPT is involved, alterations in mitochondrial ultrastructure are expected. We used electron microscope tomography to investigate at high resolution the three-dimensional (3-D) structure of rod mitochondria in retinas of wild-type and Bcl-xL mice. We analyzed more than 50 tomographic reconstructions under four conditions: wild-type control, wild-type lead-exposed, Bcl-xL transgenics and Bcl-xL lead-exposed. At a resolution of 5-7 nm, the only detectable structural modifications produced by developmental lead exposure involved the mitochondrial contact sites. No mitochondrial swelling or ruptures of membranes were observed. The surface area and density of contact sites in the apoptotic mitochondria were about twice that of the control mitochondria and the Bcl-xL mitochondria both with and without lead exposure. Coupled with our biochemical and morphological assays, this structural work shows that Bcl-xL overexpression in photoreceptors provided complete protection against developmental lead-induced, rod-selective alterations to mitochondrial function and structure. Supported by NIH Grants ES03183, EY06891, EY09213 and RR04050.

18 IMAGING OF THE MITOCHONDRIAL PERMEABILITY IN MODELS OF NEUROTOXICITY.

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Three models of chemically-induced glial, neuronal and progenitor cell-specific damage will be presented. The chemicals to be discussed produce tissue, region and/or cell-specific lesions in neural tissues. First, 1, 3-dinitrobenzene (DNB) produces a pattern of lesions in the brainstem similar in distribution and severity to those observed in the mitochondrial encephalopathies or produced by antimetabolites such as pyrithiamine and alpha-chlorhydrin. However, brainstem astrocytes are the primary target of DNB toxicity. Time-lapse confocal microscopy reveals a 10-fold difference between the sensitivity of brainstem astrocyte mitochondria to induction of the permeability transition as compared to their cortical counterparts. Second, the 2-nitroimidazole radiation-sensitizer, CI-1010, induces retinal degeneration in photoreceptor cells. Simultaneous optical imaging of the mitochondrial membrane, reactive oxygen species and cell viability permits resolution of the sequence of events leading to induction of the MPT and oxidative stress. Finally, use of confocal fluorescence microscopy enables imaging of the establishment of a mitochondrial membrane potential prior to the onset of migration from the neural crest. Challenge of neural crest cell cultures with ethanol inhibits polarization and rapidly attenuates the mitochondrial membrane potential during migration. The toxic effects of ethanol may be prevented by pre-incubation with bongkrekic acid but not cyclosporin A (CsA). The developmental significance of the inability of CsA to inhibit membrane depolarization is currently under investigation. These studies show the utility of time-lapse and real-time confocal microscopy for the *in vitro* investigation of mechanisms of neurotoxicity. (This work was supported by PHS Grant NIH-ES08846)

19 MICROSCOPY OF CELL DEATH: MITOCHONDRIAL PATHWAYS TO APOPTOSIS AND NECROSIS.

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Inhibition of mitochondrial oxidative phosphorylation progresses to uncoupling when opening of cyclosporin A (CsA)-sensitive permeability transition (PT) pores permeabilizes the mitochondrial inner membrane to small solutes. Protection by CsA against oxidative stress, ischemia/reperfusion, tumor necrosis factor-alpha exposure, Fas ligation, calcium overload, Reye-related drug toxicity and excitotoxicity implicate the mitochondrial permeability transition (MPT) in necrotic and apoptotic cell death. Confocal microscopy visualizes directly inner membrane permeabilization in single mitochondria within living cells from the translocation of impermeant fluorophores, such as calcein, between the cytosol and the mitochondrial matrix. Simultaneously, mitochondria release membrane potential-indicating fluorophores, indicating mitochondrial depolarization. Subsequently mitochondria swell, causing outer membrane rupture, and release of cytochrome c and other proapoptotic proteins from the intermembrane space. *in situ* a sequence of decreased mitochondrial NAD(P)H, increased mitochondrial free calcium and increased mitochondrial ROS formation promote the MPT and cell death. Necrotic or apoptotic cell death depends, in part, on ATP levels. If ATP levels fall profoundly, necrotic killing occurs. If ATP is maintained, apoptosis follows the MPT. The MPT also signals mitochondrial autophagy, a process that removes damaged or superfluous mitochondria. Features of necrosis, apoptosis and autophagy frequently occur together after toxic injury. A new term, necrapoptosis, describes such death processes that begin with a common stress or death signal, progress by shared pathways, but culminate in either necrosis or apoptosis depending on modifying factors such as ATP.

20 REGULATION OF ARYL HYDROCARBON RECEPTOR-MEDIATED SIGNAL TRANSDUCTION.

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The AH Receptor (AHR) is a ligand activated transcription factor that mediates many of the biological responses to halogenated and polycyclic aromatic hydrocarbons. Like many transcription factors, the AHR is a modular protein containing many distinct amino acid motifs. These motifs include i) basic regions involved in DNA binding and nuclear import, ii) a helix-loop-helix (HLH) domain involved in dimerization to ARNT and nuclear export, iii) a PAS domain involved in multiple functions such as dimerization, ligand binding and hsp90 binding and iv) a large C-terminal domain involved in transactivation function and possibly protein stability and localization. The presence of these motifs and their overlap of functions implies that the AHR has to undergo a wide range of interactions with many endogenous cellular proteins. Thus, central questions in the analysis of AHR function include how these interactions ultimately affect the magnitude and duration of AHR response to ligand exposure and in what sequence the events occur. Insight into these questions has come from the direct analysis of the AHR protein *in vitro* and *in vivo* in the presence and absence of ligand. These studies have highlighted the need to understand the mechanism of how the AHR moves through the cell and whether specific nuclear import and export domains provide areas for the cell to control AHR function and gene regulation. In addition, it has become clear that the AHR may influence cellular signaling events through interactions with other signaling pathways (for example, NF-κB, Rb etc). Therefore, it is essential to define the proteins involved in these interactions and how they might influence the traditional model of AHR-mediated signaling. Taken together, these types of studies will provide important insights into the events that impact the AHR-mediated signal transduction pathway before and after ligand binding.

21 MECHANISTIC EXAMINATION OF THE ROLE OF XAP2 IN ALTERING AH RECEPTOR SUBCELLULAR LOCALIZATION.

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The unliganded mouse aryl hydrocarbon receptor (mAhR) is a cytoplasmic, tetrameric complex consisting of the AhR ligand binding subunit, a dimer of heat shock protein 90, and the hepatitis B virus X associated protein 2 (XAP2). XAP2 increases mAhR localization in the cytoplasm. Studies were performed to determine the mechanism of cytoplasmic retention of the mAhR/XAP2 complex. In the presence of XAP2 the mAhR is retained in the cytoplasm and does not undergo dynamic ligand independent nucleocytoplasmic shuttling. One possible mechanism is through anchoring of the mAhR/XAP2 complex to the cytoskeletal matrix. This possibility was tested by disruption of both tubulin and actin networks using colchicine and cytochalasin B, respectively, no effect on the subcellular localization of the AhR complex was observed. An additional NLS(Nuclear Localization Sequence) was added to the mAhR/YFP fusion protein and fluorescence microscopy revealed that AhR/YFP/NLS was no longer retained in the cytoplasm in the presence of XAP2, suggesting that XAP2 does not tether the receptor complex. A second possible mechanism of cytoplasmic retention of the mAhR by XAP2, is through blocking access to, or altering the conformation of, the bipartite NLS. The former possibility was tested by the use of an antibody directed against the mAhR's NLS and the results indicated that the NLS is accessible even in the presence of XAP2 in the core receptor complex. Whether or not XAP2 alters the conformation of the bipartite NLS leading to reduced importin binding remains a possibility. The

human AhR (hAhR) has been shown to differ biochemically from the mAhR. The ability of XAP2 to influence localization of the hAhR was examined. Results suggest that XAP2 does not influence localization or nucleocytoplasmic shuttling of the hAhR. Additionally XAP2 was unable to enhance hAhR levels, in contrast to what has been observed with the mAhR.

22 EXAMINATION OF THE AH RECEPTOR-RETINOBLASTOMA PROTEIN INTERACTION AND IMPACT ON CELL CYCLE CONTROL.

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The aryl hydrocarbon receptor (AhR) belongs to the basic helix-loop-helix/PAS family of transcription factors that regulate critical functions during development and tissue homeostasis. Within this family, the AhR is the only member conditionally activated in response to ligand binding, typified by 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD). The novel finding that the retinoblastoma tumor suppressor protein (pRb) interacts directly with the AhR through two distinct receptor domains spurred our interest in AhR-mediated cell cycle control. We have characterized one of the binding domains. More recent evidence revealed that TCDD-induced AhR-mediated G1 cell cycle arrest in 5L hepatoma cells depends on coactivation by the pRb. The cell cycle arrest seems to require p27Kip1 and a pRb activity apparently distinct from its function as a repressor of E2F-mediated transcription. In contrast, experiments using synchronized 5L cells linked AhR activity with a proliferative response, suggestive of a more complex role for the AhR in cell cycle control. These studies identified an unexpected connection between the persistence of AhR signaling and cell cycle control.

23 IMPACT OF AH RECEPTOR DEGRADATION ON GENE TRANSCRIPTION.

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Transcriptional regulation of gene expression represents a means of control for many fundamental cellular processes, such as cell growth and differentiation, and for responses to endogenous and exogenous signals, such as the adaptive/toxic responses to environmental chemicals. As such, transcription factors that mediate gene expression to specific signals are often tightly regulated to ensure physiologically adequate gene transcription and thus, the homeostasis of the cell. The ubiquitin-26S proteasome-mediated proteolysis has been implicated in the regulation of various types of cellular proteins. In this study, we analyzed the mechanism of agonist-induced degradation of the Ah receptor and its functional impact on AhR-mediated gene transcription to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD). Biochemical and genetic data reveal that TCDD induces ubiquitination of AhR and shortening of the half-life of the protein through the 26S-proteasome mediated protein degradation. The agonist induced AhR degradation can be blocked by using inhibitors of the 26S proteasomes (MG123 and lactacystin) or cycloheximide, a potent inhibitor of protein synthesis; these data implicate a labile factor in controlling the degradation of AhR, which we designated Ah receptor degradation promoting factor (ADPF). Furthermore, inhibition of the AhR degradation by inhibitors of the 26S proteasomes or protein synthesis markedly enhances the induction of CYP1A1 by TCDD, a phenomenon termed superinduction; these findings suggest that the agonist-induced, ADPF-mediated degradation of AhR serves as a mechanism by which gene transcription by AhR is negatively controlled in cells. This view is supported by the observation that inhibition of the AhR degradation by either MG132 or cycloheximide superinduces a number of other AhR target genes, including TiPARP, a novel TCDD-inducible poly(ADP-ribose) polymerase. Our findings provide new insights into the control of the activity of agonist activated AhR through a regulated, proteasomal protein degradation pathway. Our current research is aimed at cloning of ADPF.

24 DIRECT ANALYSIS OF THE COMPLEX RELATIONSHIP BETWEEN NUCLEAR EXPORT AND AH RECEPTOR-MEDIATED GENE REGULATION.

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The AH receptor (AHR) is a modular protein containing distinct amino acid motifs that confer function to the receptor. Studies have revealed that the AHR contains both nuclear localization (NLS) and nuclear export (NES) signals that control in part, the subcellular location of the AHR and may influence the ability of the AHR to be activated. Thus, studies were performed to examine the relationship between the nuclear export of the AHR and AHR-mediated gene regulation. Blockage of nuclear export in human HepG2 cells with leptomycin B (LMB) resulted in a ligand-mediated increase in the level of AHR•ARNT complex in the nucleus and correlate reductions in agonist stimulated AHR degradation. However, despite the presence of high levels of the AHR•ARNT dimmer, induction of numerous AHR-responsive reporter genes and endogenous *CYP1A1* was reduced by 78-89%. To determine whether there was a direct relationship between blockage of export of the AHR and the reduced levels of gene regulation, stable cell lines were produced that expressed an AHR with a mutant NES. Immunohistochemical and biochemical analysis of these cells and direct comparison to cells expressing a wild type AHR protein showed that mutation of the NES *did not* affect the ability of the cells to induce endogenous *CYP1A1* following ligand exposure. To confirm these results and evaluate the temporal aspect of the signaling pathway, wild type and NES mutant AHR protein were produced *in vitro* and then microinjected directly into the nucleus of various culture cells. While export was blocked, the injected cells induced high levels of *CYP1A1* following agonist exposure. These findings demonstrate that it is possible to generate an AHR protein defective in nuclear export that functions in agonist-mediated gene induction. This implies that the negative affect of LMB on gene induction is independent of the nuclear export of the AHR and involves a novel factor that must interact with the AHR-mediated signal transduction pathway. Supported by NIEHS grants ES08980 and ES10401

25 REGULATORY AND INDUSTRY APPROACHES TO IMMUNOTOXICOLOGY ASSESSMENT OF PHARMACEUTICALS IN EUROPE, JAPAN, AND THE UNITED STATES.

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Assessment of pharmaceuticals for potential immunotoxicity seeks to identify adverse effects such as immunosuppression and/or activation of the immune system resulting in drug allergy or autoimmunity. A variety of methods have been used to evaluate potential drug-induced alterations in immune function. The usefulness of these assays in drug development has been extensively debated. Regulatory agencies in Europe, the United States, and Japan have published, proposed, or considered guidances for immunotoxicology assessment of pharmaceuticals over the past several years. In each case, the documents reflect the consensus of opinion within the regulatory agency as to the most reasonable and effective strategy to detect the potential of new drugs to cause adverse immune system effects. There are differences in the specific testing strategies suggested and in the scope of the issues these documents seek to address. For example, the issue of potential for drug allergy is not covered in all cases, and some of the strategies for evaluating potential immunosuppression are not completely harmonious. In this roundtable session, representatives from regulatory agencies and the pharmaceutical industry will discuss immunotoxicity testing of pharmaceuticals. Areas of agreement and disagreement among the parties will be addressed to elucidate the basis for the different approaches proposed.

26 EMEA GUIDELINES.

E. Putman. *Preclinical Assessment Group of the Medicines Evaluation Board, National Institue of Public Health, Bilthoven, Netherlands.* Sponsor: J. Weaver.

The Note for Guidance on Repeated Dose Toxicity was released in July 2000 by the CPMP (www.emea.eu.int/pdfs/human/swp/104299en.pdf). This Note for Guidance was revised to update the guidance on immunotoxicity. Immune toxicity screening is incorporated in the Note for Guidance in accordance to the tiered testing approach, i.e. an initial screening phase and extended studies. The tiered testing approach is considered sufficiently reliable to be used in a regulatory setting. Tiered testing strategies have been developed to assess direct immunotoxicity (suppression or stimulation). Hypersensitivity or testing for autoimmune potential is not in the scope of this Note for Guidance. In attempting to prevent an increase in animal use and number of studies the SWP decided not to issue a separate guideline on immunotoxicity, but to update the Note for Guidance on Repeated Dose Toxicity. As a result, repeated dose toxicity test evaluation has been extended to include immunotoxicity screening. The initial screening phase is designed to enhance the sensitivity of standard toxicity testing for immune toxic effects, preferably without the use of satellite animals. The NK-cell activity assay and lymphocyte subset phenotyping are selected because they fulfil these criteria. As an alternative, the primary antibody response to T-cell dependent antigen is suggested which allows a more holistic screen of the immune system. For the interpretation of the initial immunotoxicity screen the CPMP document advocates an integrative analysis of the changes in the immune system and other types of toxicity and the health status of the test animal. If the initial screening phase suggests direct immunotoxicity, follow-on studies in animals may be warranted on a case-by-case basis to further study

the altered immune response. The design of extended animal studies will depend on the nature of the immunological changes observed in the initial screening phase. It should include comprehensive *in vivo* or *ex vivo* assays of immune function. For guidance on hypersensitivity testing see (www.emea.eu.int/pdfs/human/swp/214500en.pdf).

27 CURRENT PRACTICE OF IMMUNOTOXICOLOGY TESTING IN JAPAN.

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Sponsor: J. Weaver.

Immunotoxicology studies of pharmaceuticals have been under intensive discussion for years in Japan. In most Japanese pharmaceutical companies, evaluation of unintended immunosuppressive effects of drugs is initiated by the results of standard nonclinical toxicology studies and/or their pharmacological effects. In the standard nonclinical toxicology studies, lymphoid organ weights are measured, and hematological and histopathological findings are collected. Cellularity of the spleen and bone marrow, distribution of lymphocyte subsets, antibody response to a T-cell dependent antigen, lymphocyte blastogenesis, mixed lymphocyte reaction, NK cell activity, delayed-type hypersensitivity and CFU-GM have been additionally examined in several companies. These parameters were selected based on the immunotoxicological features of test compounds. Antigenicity studies are widely conducted. Considering the genetic restriction of immune responses against low molecular weight compounds, it is very difficult to reproduce the immune responses of all humans, who have different genetic backgrounds, in animal experiments. Japanese pharmaceutical companies, being aware of the limitation of the study, send the results of animal experiments to clinical doctors. The philosophy of conducting antigenicity studies in Japan has not been accepted in the USA and Europe. There are two guidelines concerning immunotoxicology testing in Japan, which are guidelines for skin sensitization studies and skin photosensitization studies. Drugs applied to the skin are tested under these guidelines. The former guideline includes several tests such as maximization and Buehler tests. I anticipate that local lymph node assay will be added in the near future. The latter guideline includes several methods such as Harber and Morikawa methods. Japanese pharmaceutical companies are attempting to set the best procedures for immunotoxicological evaluation, and insisting that this subject should be internationally discussed especially since the guidance (or the draft guidance) documents for immunotoxicology studies were issued from the EMEA and the FDA.

28 REGULATORY CONSIDERATIONS FOR IMMUNOTOXICOLOGY ASSESSMENT OF INVESTIGATIONAL DRUG PRODUCTS IN THE UNITED STATES.

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A functional immune system is vital to human survival. Assessment of adverse effects on the immune system is therefore important in the overall nonclinical evaluation of drug toxicity. Evidence of immunotoxicity can usually be observed in standard nonclinical toxicology studies, but in some cases additional studies are important. The purpose of this presentation will be to discuss the recently published Draft Guidance for Industry entitled "Immunotoxicology Evaluation of Investigational New Drugs" (www.fda.gov/cder/guidance/3010dft.htm). The discussion will center on the parameters that should be routinely assessed in toxicology studies to determine potential effects of investigational new drugs on immune function, cases when additional specific immunotoxicity studies should be conducted, and when additional mechanistic information could help evaluate the significance of a given drug's effect on the immune system. The utility and use of the recently accepted local lymph node assay to detect potential contact sensitizers will also be discussed along with the utility of other assays designed to test various aspects of the immune system including immunosuppression, antigenicity, hypersensitivity, autoimmunity and adverse immunostimulation.

29 TIMING, DATA INTERPRETATION AND EXPERIENCE WITH THE IMMUNOTOXICITY ASSESSMENT OF NEW DRUG CANDIDATES: A PHARMACEUTICAL INDUSTRY VIEW.

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Recently the CPMP has adopted and the FDA drafted, guidelines for the immunotoxicity assessment of pharmaceuticals. This has resulted in much discussion in the industry concerning the timing, choice and validity of methods proposed and the need for such testing of new drug candidates (NCEs). During the past 15 years several inter-laboratory evaluation studies (e.g., NIEHS, IPCS, BGA, and privately sponsored) were conducted to optimize and evaluate methods and approaches using reference compounds in rodents in order to define their predictive value for detecting toxicity to the immune system. These published studies provided the basis for the CPMP and pending FDA Guidelines. Knowing that regulations were probably forthcoming, we at Sanofi-Synthelabo (SSL) have applied immunotoxicity methods to evaluate new drug candidates during pre-clinical development for the past 10 years. SSL has evaluated more than 25 NCEs from multiple therapeutic classes as well as reference compounds (azathioprine, dexamethasone, cyclophosphamide and cyclosporin A). Our experience and recommendation relative to timing of the studies, the use of multiple animal species, the interpretation of the various immunotoxicity assays and the outcome from the evaluation of NCEs and reference standards will be discussed. Our experience has led us to believe that immunotoxicity parameters may represent an important adjunct for the safety assessment of NCEs, that these methods must be carefully integrated into the drug development process, and yield an unexpectedly low frequency of positive results. The decision to do an immunotoxicity evaluation should probably be driven by a pathologic or clinical hematology finding, by the drug's indication or chemical class, or the indication for which the NCE is being evaluated (e.g., anti-Aids NCE) on a case-by-case basis.

30 *IN VIVO* FORMATION OF CERAMIDE-LIKE *N*-ACYLATED AMINOPENTOLS FROM HYDROLYZED FUMONISIN B_1.

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Fumonisins are toxic and carcinogenic mycotoxins found in corn-based foods. Alkaline conditions such as those used for making masa from corn convert fumonisins to their hydrolyzed forms. The subchronic liver and kidney effects of hydrolyzed fumonisin B_1 (HFB_1), also referred to as aminopentol (AP_1), and fumonisin B_1 (FB_1) in rodents are similar and HFB_1, like FB_1, inhibits the enzyme ceramide synthase. Fumonisin metabolism has not been shown. However, formation of the ceramide-like compound *N*-palmitoyl aminopentol$_1$ (PAP_1) from palmitoyl-CoA and HFB_1 by rat liver microsomes *in vitro* has recently been reported. PAP_1 was more toxic (using cell death as the endpoint) than FB_1 or HFB_1 to cultured HT29 human colonic cells. To determine if PAP_1 or other *N*-acylated aminopentols (AAP_1) are formed *in vivo*, male rats (n=3) were given 52, 115 or 230 HFB_1/day (ip injection) for 5 days. Controls (n=2) received the saline vehicle. Two rats/group were euthanized 2 hours after the last dose. Remaining animals were killed 3 weeks later. HFB_1 was not toxic and did not cause microscopic kidney or liver lesions. It did, however, partially inhibit ceramide synthase, as shown by increased sphinganine and sphingosine concentrations in liver and kidney. Mass spectrometric analysis revealed a series of AAP_1 compounds in liver of the HFB_1-exposed rats. The metabolites' acyl groups ranged in length from C_{16} (PAP_1) to C_{24}. C_{24} compounds were most abundant, ranging from 0.5-2.4 nmole/g tissue, while up to 0.3 nmole/g of the other metabolites were found. Lesser amounts were detected in kidney and blood. The AAP_1 metabolites were not persistent; none were detected 3 weeks after the final dose. These findings demonstrate that HFB_1 is a substrate for ceramide synthase *in vivo*. The effects of AAP_1 metabolites in animals chronically exposed to HFB_1 warrant investigation.

31 FUMONISIN B_1 (FB) ALTERS OUABAIN-INSENSITIVE BUT NOT OUABAIN-SENSITIVE ^{86}RB UPTAKE IN LLC-PK_1 CELLS WITHOUT ALTERING MEMBRANE PERMEABILITY.

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FB is a fungal inhibitor of sphingolipid biosynthesis found in corn worldwide. Inhibition of ceramide synthase causes an increase in free sphingoid bases and depletion of glycosphingolipids and sphingomyelin. Free sphingoid bases can modulate Na+/K+ ATPase activity and altered glycosphingolipid expression can modulate plasma membrane ion permeability and other membrane functions. FB also causes lipid peroxidation in rat hepatocytes and cultured cells. The purpose of this study was to determine if FB can alter membrane ion permeability, Na+/K+ ATPase function, or induce lipid peroxidation in LLC-PK1 cells. Altered membrane ion permeability and Na+/K+ ATPase function was assessed by comparing 86Rb (a transport analog for potassium) efflux and ouabain-sensitive and ouabain-insensitive 86Rb uptake in sub-confluent LLC-PK1 cells exposed to 20 µM FB (a minimally cyto-

toxic concentration) for 72 h. Lipid peroxidation was assessed using the thiobarbituric acid assay in cultures grown under similar conditions but in growth medium without phenol red and at 50 μM FB (a cytotoxic concentration). The results show that 20 μM FB for 72 h had no effect on 86Rb efflux from 86Rb loaded LLC-PK1 cells nor was there any increase in thiobarbituric acid substances at 50 μM. These findings are consistent with the conclusion that under these conditions FB had no effect on membrane permeability. There was also no significant effect on ouabain-sensitive 86Rb uptake indicating that FB had no effect on Na+/K+ ATPase function. There was however a significant inhibition in ouabain-insensitive 86Rb uptake indicating that a minimally cytotoxic but ceramide synthase inhibitory concentration of FB can modify potassium transport processes. A possible target is the ouabain-insensitive phospholipid-dependent 86Rb uptake associated with transporters such as that of the non-gastric H+/K+ ATPase.

32 FUMONISIN B1 INDUCES DECREASED EXPRESSION OF P42 MAP KINASE IN LLC-PK1 CELLS.

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Fumonisin B1 (FB1) is a fungal toxin produced by *Fusarium verticillioides*, a common pathogen of corn. FB1 inhibits ceramide synthase, a key enzyme in the *de novo* sphingolipid biosynthesis and turnover pathways. Free sphingoid bases, sphingoid base metabolites, ceramide and more complex sphingolipids generated in the biosynthesis and turnover pathways modulate many downstream signals including mitogen-activated protein (MAP) kinases. In LLC-PK1 (porcine renal proximal tubule epithelial) cells, FB1 inhibits cell proliferation, induces apoptosis, and alters cell-cell contact in a time and concentration-dependent manner. The increased apoptosis and decreased cell proliferation can be prevented by ISP-1, an inhibitor of serine palmitoyltransferase, the first and rate-limiting enzyme in *de novo* sphingolipid biosynthesis. However, the downstream signaling pathways that are affected by FB1 disruption of sphingolipid metabolism are not well understood. The purpose of this study was to determine, in LLC-PK1 cells, changes in expression of p42 MAP kinase, also called extracellular signal-regulated kinase 2 (ERK2) in response to a FB1 concentration known to inhibit cell growth and induce increased apoptosis. Significant inhibition of cell growth was first noted after 48 h exposure to FB1 (50 μM). However, p42 MAP kinase was decreased at 24 h and at all subsequent time-points (48 and 72 h) relative to the concurrent control. FB1 treatment in the absence of serum further reduced the expression of p42 at 48 and 72 h. In order to determine if decreased p42 expression was due to FB1-induced elevation in free sphingoid bases, cells were treated with a combination of ISP-1 and FB1. ISP-1 did not reverse the decreased expression of p42 caused by FB1. However, ISP-1 alone also caused a decreased expression of p42, indicating that FB1-mediated changes in expression of p42 could be independent of alterations in sphingoid bases but dependent on *de novo* sphingolipid biosynthesis.

33 EFFECT OF FUMONISIN B_1 ON PROTEIN KINASE C-α IN CULTURED RENAL LLC-PK_1 CELLS.

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Fumonisin B_1 (FB_1) is a ubiquitous, potent, and naturally occurring mycotoxin produced by the fungus *Fusarium verticillioides*. It has been implicated in fatal and debilitating diseases in both animals and humans. FB_1 is known to induce toxicity by disruption of *de novo* sphingolipid biosynthesis *via* inhibition of ceramide synthase and induction of numerous proinflammatory cytokines, including tumor necrosis factor α. Protein kinase C (PKC) is a serine/threonine kinase involved in a number of signal transduction pathways, including apoptosis and carcinogenesis. As sphingolipids are the natural inhibitors of PKC, it is conceivable that FB_1 may affect PKC-regulated functions. FB_1 has been shown to cause both activation and repression of PKC depending on the dose, cell type and PKC isoform. This study investigated the acute temporal and dose effects of FB_1 on PKC isoforms in pig renal epithelial cells (LLC-PK_1). Exposure of LLC-PK_1 cells to 1 μM FB_1 caused a rapid and transient translocation (5 min) of PKCα from the cytosol to the plasma membrane. A time-dependent increase in the stabilization of cytosolic PKCα (15-30 min) was followed by a decrease at 120 min. PKC isoforms -δ and -ε were unaffected by FB_1 at all time periods. PKCα membrane translocation increased in a dose-dependent manner with a significant increase at 0.3 μM and 1 μM FB_1. Subsequent inhibition of PKCα at FB_1 concentrations ≥ 3 μM FB_1 was observed. Pretreatment of LLC-PK_1 cells with the PKC activator, phorbol 12-myristate 13-acetate (PMA) resulted in a further increase in PKCα translocation as compared to PMA alone. The FB_1-induced PKCα translocation was reversed by pretreatment of LLC-PK_1 cells with 100 nM Calphostin C, an inhibitor of PKC. The serine palmitoyl transferase inhibitor, myriocin, did not prevent the FB_1-induced translocation of PKCα. The ability of FB_1 to induce PKCα translocation in LLC-PK_1 cells and ultimately its signal transduction pathways may have implications in its ability to produce both apoptosis and carcinogenesis. (Supported in part by NIH grant ES09403).

34 SERUM BIOCHEMICAL AND LIPID CHANGES IN SINCLAIR MINIPIGS FED LOW LEVELS OF FUMONISIN B1 FOR SIX MONTHS.

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The fumonisin mycotoxins are common contaminants of corn. A major concern for humans ingesting fumonisin contaminated food is the cardiotoxicity and hypercholesterolemia observed in swine and nonhuman primates. Fumonisin induced hypercholesterolemia has been identified in all species, and in swine at doses as low as 1 ppm fumonisin B1 (FB1). To further examine the Dose-Response relationship of FB1 induced hypercholesterolemia and changes in lipid profile, 35 Sinclair minipigs (barrows, 7 to 14 wk of age) were fed FB1 at 0, 0.5, 1.0, 2.0 or 10.0 ppm (7 pigs/group) for 6 months. Purified FB1 was obtained from the FDA. FB1 containing feed was mixed in 10 kg premix batches and diluted with mycotoxin free base diet to 10 ppm FB1. Pigs were weighed and blood obtained by jugular venipuncture after 24 hr of fasting; weekly for the first 6 wk and then every 2 wk. Hepatic enzyme activities and concentrations of serum cholesterol, triglycerides, high and low density lipoproteins, bilirubin, bile acids, blood urea nitrogen, creatinine and electrolytes were determined. Complete blood counts also were performed. At wk 18, since no changes were observed in the parameters listed above, the dose in the high dose group was increased from 10 to 30 ppm FB1. No significant differences were observed in serum biochemistry or lipid parameters in fumonisin treated pigs, compared to control pigs, during the 6 month study. This is in contrast to previously reported hypercholesterolemia in pigs fed 1 ppm FB1 for 8 weeks (Rotter et al., 1996); however, Yorkshire pigs were used, and hypercholesterolemia did not occur in swine fed FB1 at 10 ppm. Based on this study in which doses bracketed the FDA recommended maximum levels of 2 to 4 ppm FB1, concern regarding potential FB1 induced atherosclerotic effects does not appear warranted. Supported by USDA-CSREES grant #928-39453, FDA and ILSI.

35 SPHINGOLIPID CHANGES AND PATHOLOGY IN SINCLAIR MINIPIGS FED LOW LEVELS OF FUMONISIN B1 FOR SIX MONTHS.

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The fumonisin mycotoxins disrupt sphingolipid biosynthesis resulting in increased levels of sphingosine (So) and sphinganine (Sa) in tissue and serum. This appears to be the first detectable fumonisin induced biochemical change in swine. To examine the fumonisin B1 (FB1) induced Dose-Response of So and Sa and correlate it with morphologic changes in a low dose long term study, 35 Sinclair minipigs (barrows, 7 to 14 wk of age) were fed FB1 at 0, 0.5, 1.0, 2.0 or 10.0 ppm (7 pigs/group) for 6 months. Purified FB1 was obtained from the FDA. FB1 containing feed was mixed in 10 kg premix batches and diluted with mycotoxin free base diet to 10 ppm FB1. At wk 18, since no changes were observed in biochemical or lipid parameters, the highest dose was increased from 10 to 30 ppm FB1. At study end, pigs were weighed, euthanized and necropsied. Major organs were weighed and samples of all major tissues and arteries fixed in 10% formalin. Samples for sphingolipid analysis were frozen in liquid nitrogen and kept at - 80 C until Sa and So were determined by HPLC. Fumonisin induced gross or histological alterations were not observed, nor did absolute organ weights or organ to body weight ratios differ among groups. In the highest dose (10 to 30 FB1 ppm) group, Sa and So concentrations were increased in kidney, heart, aorta, and urine; Sa was increased in liver and lung; while neither Sa nor So were increased in brain. These results, as well as the lack of serum biochemical and lipid changes in the same study (see sister abstract), support the earlier assumption that changes in sphingoid bases are the earliest detectable biochemical changes induced by FB1, and appear to indicate the NOEL for FB1 is more than 2 ppm, which is more than the FDA proposed regulatory limit of 1 to 2 ppm. Supported by USDA-CSREES grant #928-39453, FDA and ILSI.

36 MOLECULAR SIGNATURE OF DIOXIN IN MURINE VASCULAR SMOOTH MUSCLE CELLS: ROLE OF THE ARYL HYDROCARBON RECEPTOR IN CARDIOVASCULAR DISEASE.

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Cardiovascular disease, primarily associated with disorders of atherosclerotic etiology, is the main cause of morbidity and mortality in the United States. Overt and cumulative exposure to environmental or occupational toxicants may play a role in the onset and progression of this disease. More than 35 different predisposing risk

factors for atherosclerosis have been identified, but little is known of the mechanism of action of environmental agents, due in part to lack of epidemiological studies and in part to lack of adequate animal models. Dioxins, such as 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), have been implicated as risk factors for atherosclerosis in humans. The objective of these studies was to test the hypothesis that TCDD exposure results in an aryl hydrocarbon receptor (AHR)-dependent reprogramming of gene expression in vascular smooth muscle cells (vSMCs). To test this hypothesis, we used gene arrays to examine genes involved in cell cycle regulation and apoptosis, as well as cytokines, chemokines and their receptors. Numerous cell cycle and apoptotic genes were found to be deregulated by 1nM TCDD in quiescent and synchronized vSMCs from *Ahr*$^{+/+}$ mice, but not in vSMCs from *Ahr*$^{-/-}$ mice. TCDD increased the mRNA levels of p21$^{Cip1/Waf1}$, mdm2, caspase 1, and cyclin D1 compared to controls by 2.7, 2.4, 2.2, and 1.8 fold, respectively. Decreases in TCDD-mediated gene expression were observed for p15^{Ink4b} and p57^{Kip2} compared to controls by 2.7 and 2.3 fold, respectively. At the protein level alterations in expression were detected for mdm2, caspase 1, and p15^{Ink4b}. These data suggest that vascular toxicants, such as TCDD, may alter gene profiles in vSMC in AHR-dependent manner that can translate into functional changes at the protein level. These finding may help us define a molecular fingerprint of vascular toxicants of utility to reduce the use of animals in toxicity testing. (Supported by Colgate-Palmolive/Society of Toxicology Postdoctoral Fellowship and NIH F32ES11250-01).

37 ARYL HYDROCARBON RECEPTOR (AHR) NULL MICE EXHIBIT HYPERTENSION AND INCREASED PLASMA ENDOTHELIN LEVELS.

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AhR is a ligand-activated transcription factor known to mediate the toxicity of compounds such as 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), polychlorinated biphenyls and polycyclic aromatic hydrocarbons. Upon activation, AhR dimerizes with the aryl hydrocarbon nuclear translocator protein (ARNT). This complex then translocates into the nucleus where it binds to dioxin response elements (DREs) and upregulates a series of xenobiotic metabolizing enzymes. ARNT also heterodimerizes with hypoxia inducible factor 1 alpha (HIF1a), which then binds to hypoxia responsive elements (HREs), increasing transcription of hypoxia-induced genes. We have shown that AhR null mice develop cardiac hypertrophy by 5 months of age and exhibit increased HIF1a signaling in the heart. In addition, endothelin-1 (ET-1) is upregulated by hypoxia and the HIF1a signaling pathway. ET-1 is a circulating peptide associated with hypertension and cardiac hypertrophy. Thus, we tested the hypothesis that AhR null mice exhibit hypertension associated with increased plasma ET-1. Catheters were surgically placed in the femoral artery of both wildtype C57Bl6 and AhR null mice, age 4 -6 months. Mean arterial blood pressure (MAP) was averaged over a 10 minute recording period on day 3 post surgery and then the mouse was sacrificed and blood collected. Plasma ET-1 was measured using an RIA. AhR null mice exhibited significantly higher MAP compared to wildtype (wildtype, 98.9 ± 1.9; null, 128.2 ± 3.0 mm Hg; $p < 0.001$). AhR null mice also exhibited significantly higher plasma ET-1 compared to wildtype (wildtype, 8.4 ± 1.1; null, 11.3 ± 0.7 fmol/ml; $p < 0.04$). Thus, AhR null mice exhibit hypertension associated with increased plasma ET-1 levels. These data suggest that the increased hypertension afterload and increased plasma ET-1 could contribute to cardiac hypertrophy in AhR null mice. Supported in part by NIH grant ES-10433 and American Heart Association Desert Mountain Affiliate 0151511Z.

38 TOWARD MECHANISMS OF 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN CARDIOVASCULAR EMBRYOTOXICITY: IDENTIFICATION OF CANDIDATE GENES USING cDNA MICROARRAYS.

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2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a powerful teratogen that perturbs the developing cardiovascular system of zebrafish. In embryos exposed to TCDD, the heart is distended and beats weakly, circulation is greatly reduced, and there is pericardial and yolk sac edema and regional hemorrhage. The molecular changes responsible for such cardiovascular embryotoxicity have yet to be defined. To address this problem, we developed zebrafish cDNA microarrays consisting of nearly 5, 200 adult heart cDNA clones, known heart markers, nuclear receptors and cytochrome P450s. Using these arrays, we have characterized alterations in embryonic gene expression resulting from TCDD exposures ranging from ED10 to ED100 for cardiovascular symptoms. Induction of CYP1A was consistently the strongest, most sensitive response. Expression of CYP19A (aromatase), AhR2, and ARNT also increased significantly after all TCDD exposures. Finally, more than fifty novel transcriptional responses were identified from among the adult heart cDNAs. By correlating the magnitude of transcriptional changes with that of toxicity, we have generated a list of genes that are likely to be involved in cardiovascular embryotoxicity. NIH #P42-ES07381

39 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) INDUCES FUNCTIONAL ACTIVATION OF THE GAMMA-INTERFERON ACTIVATED SEQUENCE IN RAT HEPATIC ENDOTHELIAL CELLS.

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The ubiquitous environmental contaminant 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a developmental toxicant, potent tumor promoter in mice, and putative human carcinogen. TCDD elicits effects through altering gene expression in susceptible cells. We have found that exposure to TCDD results in activation of the interferon-gamma (IFN-g) activated sequence (GAS). Binding of IFN-g to its cellular receptor initiates tyrosine phosphorylation of STAT proteins, dimerization and subsequent translocation to the nucleus through interactions with a combination of second-messenger molecules, resulting in activation of GAS elements in the promoter regions of IFN-g-sensitive genes. Activation of GAS elements plays an important role in modulating expression of an array of genes important in inflammation. We have found that, similar to IFN-g, TCDD induces expression of type 2 nitric oxide synthase, type II MHC and COX 2 in rat hepatic endothelial cells. The toxicity of TCDD is mediated largely through binding of the environmental toxicant to the Ah receptor. We have found that the TCDD-bound Ah receptor directly interacts with STAT 1a modifying its activity. Moreover, in a manner similar to that of IFN-g, TCDD-mediated induction of gene expression results in functional alterations to hepatic endothelial cells. Nitric oxide production and prostaglandin release were modified in endothelial cells following TCDD-treatment. Through up regulation of STAT 1a, NOS2, type II major histocompatibility antigen, cyclooxygenase2 and other proinflammatory proteins, endothelial cells become immunologically activated, a process that plays an important role in inflammation and tissue injury. Our findings suggest that TCDD functions in endothelial cells by interacting in the IFN-g signaling pathway. We speculate that IFN-g mimetic activities, mediate, in part, the toxicity of TCDD in the liver. Supported by NIH ES 06897.

40 ARYL HYDROCARBON RECEPTOR (AHR) NULL MICE SHOW INCREASED CARDIAC HYPOXIA SIGNALING, BUT NO INCREASE IN CARDIAC HYPOXIA.

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2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) has been shown to cause cardiac malformations in the chick embryo and these effects are likely mediated by the AhR. Upon TCDD binding, AhR dimerizes with AhR nuclear translocator (ARNT), binds dioxin response elements, and upregulates xenobiotic metabolism genes. In response to hypoxia, ARNT also heterodimerizes with hypoxia-inducible factor 1 alpha (HIF1a). The HIF1a/ARNT complex then binds to hypoxia responsive elements, and facilitates transcription of hypoxia-responsive genes such as vascular endothelial growth factor (VEGF). This suggests that cross-talk between the TCDD and hypoxic signaling pathways could exist within the heart. We have previously reported that AhR null mice develop cardiac hypertrophy by 5 mo, as measured by increases in heart weight, heart/body weight ratio, and the cardiac hypertrophy markers, beta-myosin heavy chain and myosin light chain-2V. Here we show that AhR -/- mouse hearts have increased HIF1a protein and upregulate the hypoxia-responsive gene VEGF at 5 mo. We then used ^{3}H-misonidazole (MISO), which binds tissues in a pO_2-dependent manner, to determine the relative oxygen concentrations in wildtype and AhR -/- mouse hearts. At 3 mo, a time when no significant increase in heart weight nor HIF1a protein is seen in AhR -/- mice, no increase in MISO binding was observed. At 5 mo, when AhR -/- mice show a significant increase in both heart weight and HIF1a protein, there was still no increase in cardiac MISO binding. These data show that AhR -/- mice develop a significant increase in signaling through the cardiac HIF1a-mediated pathway despite normoxic conditions within the heart. This suggests that the increase in cardiac HIF1a signaling may be caused by disregulation of the HIF1a pathway due to a loss of AhR within the heart, and not by impaired oxygenation of the myocardium. Supported by NIH ES10433 and AHA Desert Mountain Affiliate 0151511Z.

41 GLOBAL GENE EXPRESSION PROFILES IN CHEMICAL ATHEROGENESIS.

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Environmental hydrocarbons have been implicated as significant risk factors in human atherosclerotic vascular disease. Using benzo(a)pyrene (BaP) as a model compound, this laboratory has shown that interference with gene transcription is a critical event in the induction of proliferative (i.e atherogenic) phenotypes in vascular smooth muscle cells (vSMCs). The complexity of the cellular response elicited by BaP requires characterization of interactive gene networks involved in the atherogenic response. To achieve this objective, vSMCs isolated from the thoracic aorta of adult C57BL6J were established in serial culture and used as a model to evaluate the atherogenic response to BaP. G0 synchronized cultures were released into growth by addition of fetal bovine serum in the presence of DMSO (control) or 3 uM BaP for 8 hr, a regimen known to modulate vSMC phenotypes *in vitro*. Control and treated mRNAs were isolated and labeled with Cy3 and Cy5, respectively, and hybridized to custom-made mouse cDNA microarrays of 960 genes spotted in triplicate. Array intensities were evaluated as prescribed by GeneSpring® software to identify differentially expressed genes. Over 150 genes were altered in vSMCs by BaP treatment. Genes coding for RAS, cholesterol-regulated CR36 protein, several heat shock proteins, retinoic acid repressible protein, and insulin-like growth factor 1 receptor were significantly upregulated. Other genes increased by atherogen treatment included peroxiredoxin, cofilin, bromodomain PHD finger transcription factor, TIF1 beta, GTP-binding protein, G protein gamma-2 subunit, recA /RAD51, and BAF53a. These results confirm previous findings and implicate genes involved in ras signaling, oxidative stress, DNA repair, and chromatin remodeling in the atherogenic response elicited by BaP. (Supported by NIH grants ES 04849 and ES09106. CDJ is a postdoctoral fellow in NIEHS training grant ES 07273).

42 IMMUNOMODULATORY EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN IN LUNG CELLS AND/OR TISSUE.

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Interferon (IFN) can play a role in development of primary tumor and shape tumorigenicity. Previous results using real-time RT-PCR and microarray analysis of human peripheral lung airway epithelial cells (HPL1A), showed repression of several IFN regulated genes and induction of IFN regulatory factor 4 (IRF4) when treated with 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD). We hypothesize that TCDD alters the response of HPL1A cells to interferon. Since IFN-gamma is known to inhibit cell proliferation, cells were treated with 1X104 units/ml for 24 hours plus or minus TCDD (10nM) pretreatment. An aqueous soluble tetrazolium/formazan assay was used to detect cell growth changes. Up to 30 nM concentrations, TCDD did not cause any cell growth changes compared to cells given vehicle (FBS) only. By comparison, treatment with INF-gamma caused a 28 % decrease in cell growth ($P < 0.001$). Pre-treatment with TCDD (10nM), reduced this decrease in cell growth caused by INF-gamma by approximately 50 % ($P < 0.001$). Our next objective was to do a cross-species comparison looking at *in vivo* gene expression in rat lung. We sought to determine how TCDD treatment affected interferon regulatory factor 4 (IRF4) and myxovirus (influenza) resistance 1, homolog of murine interferon-inducible protein p78 (MX1). Sprague-Dawley rats were treated by oral gavage twice weekly. Control animals were given vehicle (corn oil) only, and the treated group consisted of a subacute treatment (350 ng/kg twice a week). Using RNA isolated from rat lung tissue, we analyzed IRF4 and MX1 expression by real-time RT-PCR. We found that IRF4 was induced after 5 weeks TCDD treatment, while MX1 was suppressed. This inverse gene relationship was the trend of expression we previously found in the HPL1A cell line. Overall, this data suggest a novel role for TCDD in immunomodulation in human lung cells that appears to be conserved in rats

43 MATRIX-DEPENDENT ALTERATIONS IN REL PROTEIN EXPRESSION AND NF-KAPPAB ACTIVITY AFTER OXIDATIVE INJURY.

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Repeated cycles of chemical injury by allylamine induce proliferative (i.e. atherogenic) vascular smooth muscle cell phenotypes in male rats. Activated smooth muscle cells display matrix-specific changes in proliferation that correlate with NF-kappaB binding activity. Injured cells exhibit a proliferative advantage when seeded on plastic, fibronectin, or laminin, but not collagen. These cells also display altered patterns of osteopontin expression, secretion, and cleavage, coupled with changes in the expression of several integrin subunits. Integrin-mediated increases in NF-kappaB activity may occur as a result of altered expression profiles of NF-kappaB constituent proteins. To test this hypothesis, the present studies examined changes in Rel protein expression in populations of control and oxidatively stressed cells following repeated cycles of allylamine injury. Sprague-Dawley rats (175-180 g) were gavaged once daily with allylamine (70 mg/kg) for 20 consecutive days. Vascular smooth muscle cells were isolated by enzymatic digestion, and cells maintained in serial culture for up to 25 passages. Western analysis demonstrated that Rel-A protein levels decreased in allylamine cells seeded on plastic, with concomitant appearance of a novel 50kDa immunoreactive protein. Furthermore, p50 protein levels increased in allylamine cells seeded on plastic, but not on collagen, fibronectin, or laminin. Seeding of allylamine cells on plastic, but not collagen, fibronectin, or laminin reduced Rel-B protein levels relative to controls. These results suggest that changes in Rel protein expression may contribute to the expression and/or maintenance of allylamine-induced atherogenic phenotypes. (This work was supported in part by NIH grants HL62539 and ES09106).

44 TOXICOGENOMICS: FINGERPRINTING TOXIC COMPOUNDS USING GENE EXPRESSION PROFILES.

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In the recent past there has been considerable interest in linking the modulation of gene expression to the toxicity of drugs. Toxicogenomics is considered a valuable tool for the understanding of underlying mechanisms of toxicity and for the early prediction of toxic liabilities in toxicological studies. It is expected that gene expression profiles will generate fingerprints typical of classes of toxicants, as well as provide new and sensitive toxicity markers. In this paper we established and investigated the effect of five hepatotoxic compounds with a similar mechanism of toxicity on gene expression profiles in male Wistar rats. The so-called "direct acting compounds" tested in this study were Thioacetamide, Bromobenzene, CCl4, dichlorobenzene and hydrazine. The gene expression in the liver was evaluated using Affymetrix GeneChip® microarrays. Genes regulated by at least 4 of these compounds were considered characteristic for these toxins. This "direct acting fingerprint" was then compared with the modulation of the same genes caused by four steatotic compounds (Tetracycline, Amiodarone, Doxycycline and Amineptine) and by 1, 4-DCB, a non-toxic regioisomer of the "direct acting" compound 1, 2-DCB. The results presented in this paper show that gene expression profiles can be used to distinguish "direct acting" compounds from other types of compounds such as the non-toxic compound 1, 4-DCB and the tested steatotic compounds. Furthermore, the assessment of the modulation of gene expression in two experiments performed independently with the same compound showed good reproducibility of the gene expression profiles. In conclusion, gene expression profiles in liver after exposure to hepatotoxins are reproducible and characteristic of classes of toxins. Gene expression fingerprints allow different types of toxins to be distinguished from each other and from non-toxic compounds.

45 COMPOUND PROFILING USING CDNA ARRAY BASED DIFFERENTIAL GENE EXPRESSION OF HUMAN AND RAT HEPATOCYTES.

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Compound profiling strategies are of great interest in the process of developing new pharmaceuticals. Combinatorial chemistry and high throughput screening technologies are flooding the development pipelines with possible candidates and efficient and reliable tools are required to properly rank them. Target validation and early predictive toxicogenomics are areas which can be especially forwarded through microarray applications. We have started to set up a ToxSAYS™ database, where we are determining the gene expression of more than 1, 000 well-defined human and rat ortholog genes in response to treatment with the 150 most frequently prescribed drugs and a whole range of chemicals with known toxicological effects. For the development of this database, we investigate gene expression profiles in primary rat and human hepatocytes, and in tissues and organs from GLP-compliant four-week toxicity studies. The selected genes are involved in Toxicology-relevant cell activities like apoptosis, cell cycling, proliferation, DNA damage/repair, inflammation, oxidative stress, transport and metabolism. For each gene 200 -400 bp cDNA fragments are rigidly selected to avoid cross reactions between high homologous gene families. Starting with a set of toxic compounds e.g., Aroclor 1254, Allylalcohol, Galactosamine, Concanavaline, Acetaminophen and Thioacetamide we demonstrate the induction of distinct gene expression profiles by each of these

compounds. A correlation of gene expression profiles with a time and dose dependent mode of action is exemplified showing the significant regulation of genes which are targeted by the Ah-ARNT transcription factor in response to Aroclor 1254.

46 GENE EXPRESSION CHANGES IN RAT LIVER ASSOCIATED WITH CHOLESTASIS.

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Microarray technology enables the investigation of large scale gene expression in response to toxic insult. By generating expression data on many genes simultaneously it is feasible that compounds could be classified according to their gene expression pattern or "signature." The aim of the following work was to determine whether different mechanisms of hepatotoxicity could be distinguished based on differential gene expression analysis. Oestrogens and oestrogen glucuronides have been chosen specifically for the purpose of this study as they have long been implicated in the pathogenesis of intrahepatic cholestasis. To complement these, one structurally-related oestrogen that does not cause cholestasis and the model hepatotoxins alpha-naphthyl isothiocyanate and carbon tetrachloride have been added for comparative purposes. Male Sprague-Dawley rats, 250-335g were administered with either 17-alpha-ethinyloestradiol, estradiol-17-beta-D-glucuronide, oestriol-17-beta-D-glucuronide, oestriol-16-alpha-(beta-D-glucuronide) or oestradiol-3-beta-D-glucuronide at 5mg/kg/day. Alpha-naphthyl isothiocyanate and carbon tetrachloride were administered at 50mg/kg/day and 100mg/kg/day respectively. Compounds were administered to five animals per compound group for a series of four days followed by necropsy on day five. The liver from each animal was immediately snap frozen in liquid nitrogen and stored at -80°C. Following this, total RNA was extracted and gene expression analysis carried out using a proprietary cDNA microarray system. Six replicate microarray hybridisations were performed for each RNA sample and the data were analysed by univariate and multivariate analysis. The results showed a difference in gene expression associated with different treatment groups. We propose that this technology is useful for gene expression pattern profiling associated with different hepatotoxicants and may facilitate predictive toxicology.

47 DNA MICROARRAY ANALYSIS OF PROTOTYPE HEPATOCARCINOGENS IN PRIMARY RAT HEPATOCYTES.

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An *in vitro* study was performed using primary rat hepatocytes to evaluate the gene expression profiling of known rodent hepatocarcinogens. Adult rat hepatocytes were cultured with Matrigel and treated with 2AAF, 4AAF, bemitradine (BEM), doxylamine (DOX), methapyrilene (MET), tamoxifen (TAM), and phenobarbital (PHEN) for 48 hr at concentrations ranging from 1 to 500 µM. Doses determined to be toxic by MTT reduction and LDH leakage were not evaluated for gene expression. Messenger RNA was isolated from cultured hepatocytes and used to prepare Cy3- and Cy5-labeled cDNA probes. The probes were hybridized against Incyte RatGEM 1 microarrays, which contain approximately 7, 800 arrayed sequences. Several approaches were used to analyze the profiling data, statistical evaluations, including gene clustering, correlations with phenotypes, and pathway analysis. Characteristic P450 induction profiles were observed as 2AAF, 4AAF, BEM, MET, and TAM treatment induced CYP1A1; 4AAF, BEM, and PB treatment induced CYP2B1; and 2AAF, 4AAF, BEM, DOX, TAM and PB induced CYP3A1. Clustering the expression data with predicted carcinogenicity or genotoxicity identified several candidate molecular markers that may be predictive of carcinogenicity and of genotoxicity such as thymosin beta 4. Interestingly, clustering analysis results from *in vitro* treatment of these same compounds led to a completely different subset of genes identified after *in vivo* treatment.

48 INVESTIGATION OF THE GENE EXPRESSION PROFILES OF HEPATOTOXIC AND NON-HEPATOTOXIC THIOXANTHONE ANTI-TUMOR AGENTS USING cDNA MICROARRAYS.

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Gene array technology may prove useful for investigating molecular mechanisms and screening new drug candidates. The utility of microarrays will depend on the extent to which they can identify and predict the genes that are changed in target organs. Sanofi-Synthelabo is studying the utility of microarrays using a series of 5 thioxanthone anti-tumor agents. Within this series of DNA-damaging anti-tumor agents there are both hepatotoxic (3) and non-hepatotoxic (2) compounds. The gene expression profiles of these compounds are being compared as a means of identifying genes associated with hepatotoxicity. HepG2 human hepatoblastoma cells were exposed to each thioxanthone at a concentration that results in 20-30% cell death (MTT reduction). Cells were exposed to compound for a period of 4 hours to approximate the *in vivo* half-life of the thioxanthones. Cells were incubated for 44 additional hours (w/o compound) to mimic the time frame for appearance of hepatotoxicity *in vivo*. mRNA was isolated and radiolabeled cDNA probes were generated and hybridized to Clontech Human Toxicology II™ arrays. The expression of 144 genes was altered by the thioxanthones (≥ 1.5-fold increase or decrease over untreated controls). Genes whose expression was changed included those involved in apoptosis, cell signaling, cell cycle regulation, DNA damage and repair, stress response, and xenobiotic metabolism. Of the total list of changed genes, 10 were changed only by hepatotoxic thioxanthones (e.g., GST homolog); 17 others were changed only by non-hepatotoxic thioxanthones (e.g., ERK4; MAPK4). As expected for DNA-damaging agents, genes such as p53, WAF1/p21, GADD153, GSHPX1, c-jun, and ERCC1 were changed by all 5 thioxanthones. These results suggest there are gene expression changes specific to hepatotoxic thioxanthones. A more extensive time course analysis of gene expression is being performed to determine the molecular pathways associated with the onset of hepatotoxicity.

49 GENE EXPRESSION PROFILES OF 4-AMINOBIPHENYL AND BIPHENYL IN MOUSE LIVER.

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Arylamines such as 4-aminobiphenyl (4ABP) are human and animal carcinogens. Biotransformation results in the formation of electrophilic products that can react with DNA causing mutations leading to the development of tumors. While the genotoxicity of 4ABP is well defined, little is known about alterations that may occur in the expression of genes in target tissue. A study was initiated to profile the changes in gene expression associated with acute exposure to the carcinogen 4ABP. The noncarcinogenic structural analog biphenyl (BP) was also tested in order to assess the contribution of reactive arylamine products to changes in expression patterns. Adult male C57Bl/6 mice were given an oral dose 120 mg 4ABP or BP/kg. Controls received corn oil. After 24h, livers were harvested. RNA was isolated and used to synthesize cDNAs labeled with Cy3 or Cy 5. Labeled cDNAs from control and treated animals were competitively hybridized to a 5300 microarray created using mouse cDNA clones obtained from Research Genetics, then scanned to detect fluorescence intensities. Data were examined based on 3 replicate experiments. Initial analysis at p=0.01 identified 270 genes with 4ABP-induced changes in expression and 59 with BP. With the additional stringency of a 2 fold change in expression, 60 genes were altered with 4ABP while BP induced no effect. An alternative analysis based on a 2 fold change in expression ratio and a minimum fluorescence intensity of 200 also showed no changes with BP exposure but 4ABP-induced changes in 124 genes. There were 55 genes up regulated and 69 down regulated. These included genes related to stress responses such as MT-1, TGF*beta* and Grpl2. 4ABP and BP, differing only in the presence of an animo group at the 4-position, generated compound specific gene expression profiles. These data support the conclusion that the increased number of genes affected by 4ABP is linked to its potential for further activation and formation of electrophilic products. (Supported by ES 09812 and ES 10047).

50 ASSESSMENT OF HEPATOCYTES AND LIVER SLICES AS *IN VITRO* TEST SYSTEMS TO PREDICT *IN VIVO* GENE EXPRESSION.

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Primary hepatocytes and precision cut liver slices are two common *in vitro* systems used to predict and evaluate xenobiotic induced hepatotoxicity. Toxicogenomics has recently been used to assess changes in gene expression in a variety of *in vitro* and *in vivo* systems. However, comparisons of changes in gene expression in response to toxicant exposure *in vitro* to those observed *in vivo* have not been performed. This study was conducted in rats to assess the changes in gene expression in primary hepatocytes, precision cut liver slices and *in vivo* after exposure to known hepatotoxicants. Five representative compounds, phenobarbital (PB), carbon tetrachloride (CCl_4), Wy-14, 634, alpha-naphthylisothiocyanate (ANIT) and tacrine were selected for their broad range of mechanisms in hepatocellular response. *in vivo*, male rats (4/group) were treated with acute doses of PB (100 mg/kg), CCl_4 (1ml/kg), Wy-14, 634 (150 mg/kg), ANIT (150 mg/kg) or tacrine (30 mg/kg) and sacrificed 24 hours later. *in vitro*, hepatocytes and liver slices were incubated with

PB (0.1 mM), CCl_4 (0.3 mM), Wy-14, 634 (0.5 mM), ANIT (0.1 mM) or tacrine (0.05 mM) for 24 hours. Total RNA was extracted from the three test systems and gene expression measured using Affymetrix U34A rat GeneChips. Very few genes that change with *in vivo* treatment were observed in either of the two *in vitro* systems. However, changes in gene expression in tissue slices appeared to provide a better correlation to the *in vivo* setting than hepatocytes. Additional time points and doses (or concentrations) will be required to elucidate the true correlation between the models.

51 GENE EXPRESSION PROFILING REVEALS MULTIPLE TOXICITY ENDPOINTS INDUCED BY HEPATOTOXINS.

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Microarray technology, which examines global gene expression changes, has a huge potential to revolutionize the processes of drug discovery and development. In the current study, gene expression profiling was used to investigate the mechanisms of hepatotoxicity induced by therapeutic agents. Acetaminophen, methotrexate, methapyrilene, and phenytoin are known to cause different types of hepatotoxicity. The challenge is whether gene expression profiles can distinguish these subtypes of hepatotoxicity. Sprague-Dawley rats were orally dosed with acetaminophen (4500 mg/kg/day for 6, 24, and 72h), methotrexate (1 mg/kg/day for 1, 7 and 14 days), methapyrilene (100 mg/kg/day for 1, 3, and 7 days) or phenytoin (300 mg/kg/day for 14 days). Hepatic gene expression was assessed using gene arrays containing 700 targets. Significant changes in gene expression induced by each compound were categorized into subsets according to functionality, providing insight into the mechanisms of toxicity. For example, induction of cathepsin and other inflammatory related genes by acetaminophen and methapyrilene correlated with findings such as necrosis and inflammatory cell infiltrates. While increased microsomal enzyme expression correlated with hepatocellular hypertrophy induced by phenytoin. Although it is not possible to provide a complete interpretation of all gene changes, the current study suggests that this approach is useful in delineating subtypes of hepatotoxicity.

52 ASSESSMENT OF HEPATOTOXIN-INDUCED DIFFERENTIAL GENE EXPRESSION *IN VITRO* USING cDNA MICROARRAY TECHNOLOGY.

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Toxicogenomics is a powerful tool for investigating molecular mechanisms of toxicity and predicting adverse effects by monitoring large-scale gene expression profiles. First however, it is necessary to evaluate the data generated using microarray technology with compounds of well characterised toxicity. Hepatotoxicity, being one of the most well researched toxic endpoints, was chosen for this purpose. HepG2 cells were exposed (in triplicate) to four classic hepatotoxins; carbon tetrachloride (CCl_4), clofibrate, ethanol (EtOH) and dimethylformamide (DMF) for periods of 2h and 24h at high and low doses corresponding to 50% and 20% cell death (as determined by MTT assay) respectively. Following exposure, mRNA was isolated, ^{33}P labelled cDNA probes constructed and used to probe Atlas™ Human Toxicology II arrays (Clontech). Probed arrays were exposed to X-ray film, and the images captured and analysed using AtlasImage™ software. Data revealed relatively few changes in gene expression following 2hr exposure compared to 24hr exposure, this being more pronounced at the higher doses. Although there was some similarity in differential gene expression observed across chemicals, a number of hepatotoxin-specific gene expression changes were identified, reflecting their different mechanisms of toxicity. For example, treatment with EtOH (both concentrations) gave rise predominantly to differential expression of genes involved in metabolism (e.g. CYP3A3) and stress response (e.g.β crystallin A4), whereas CCl_4 (both concentrations) resulted in gene expression changes involved in extracellular transport (e.g. OCTN1) and cell signalling (e.g. tyrosine protein kinase receptor). Real Time PCR was used to confirm expression for selected genes. In conclusion, these results suggest that specific patterns of gene expression may be associated with different mechanisms of hepatotoxicity, which may be useful as early markers of toxicity

53 GENE EXPRESSION PROFILING OF CULTURES OF PRIMARY RAT HEPATOCYTES TREATED WITH HYDRAZINE.

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Hydrazine and its derivatives are colorless liquids used by the Department of Defense as reducing agents in aircraft and propellant systems. Various toxic effects have been associated with exposure to hydrazine, including skin irritation, convulsions, hepatotoxicity, hemolytic effects, neurotoxicity, genotoxicity and it is also a suspected carcinogen. However, there are limited data on genomic effects related to hydrazine exposure. In this study, primary cultures of rat hepatocytes were treated with 0, 25, or 50 mM hydrazine for 4 hours at 37 °C. Total RNA was isolated immediately following the exposure to determine mRNA expression profiles using the Affymetrix RatTox U34 and the Affymetrix RatGenome U34A arrays. Our results indicated that hydrazine exposure caused a significant induction of stress related genes (e.g., *hsp*70s and *hsp*40s), transcription factors such as AP-1 and Sp-1 and a moderate induction of various cellular proliferation growth factors (e.g., VEGF, HB-EGF, CYR 61 and Egr1), but a significant (more than 2-fold) down-regulation of certain phase I and phase II drug metabolizing enzymes (e.g., CYP 450s, GSTs and NQO1). In addition, a significant down-regulation of the stress related heme oxygenase gene was seen. These results will provide important data for a complete risk assessment for hydrazine and serve as a baseline for evaluating the toxicity of newly synthesized high-energy chemicals as candidate replacements for hydrazine.

54 ANALYSIS OF THE SHORT TERM EFFECTS OF TREATMENT WITH PROTOTYPE HEPATOCARCINOGENS AS ASSESSED BY DNA MICROARRAYS.

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Chronic administration of high doses of certain compounds may induce hepatocellular tumors in rodents. Gene expression profiling can be utilized to characterize transcriptional responses to such treatments and improve our understanding of the mechanisms by which hepatocarcinogens produce tumors. The objective of this study was to evaluate chemically-induced changes in gene expression that may be altered during toxicity, and to identify expression patterns that may be predictive of carcinogenicity. Male Sprague-Dawley rats were treated daily for 5 days with one of three doses of several mutagenic or non-mutagenic carcinogenic or non-carcinogenic compounds. The compounds used were bemitradine, clofibrate, phenobarbital, tamoxifen, 2-AAF, 4-AAF, doxylamine, methapyrilene, and isoniazid. A single animal in one group died, but in general, all compounds and doses were well tolerated and no other mortality was observed. No gross abnormalities were noted at necropsy. Messenger RNA was isolated from livers of each animal and used to prepare Cy3- and Cy5-labeled cDNA probes. The probes were hybridized against Incyte RatGEM 1 microarrays, which contain approximately 7, 800 arrayed sequences. Several approaches were used to analyse the profiling data, statistical evaluations, including gene clustering, correlations with phenotypes, and pathway analysis. Clustering the expression data with predicted carcinogenicity or genotoxicity identified candidate molecular markers that may be predictive of carcinogenicity and of genotoxicity. Among those genes whose expression may predict carcinogenicity was TSC-22, a TGF-beta regulated gene encoding a leucine zipper that may be involved in developmental differentiation. Additional insight into the mechanisms of toxicity of some of the individual compounds was obtained through pathway analysis of genes affected by different dose levels of individual compounds.

55 TRANSCRIPTOMIC AND PROTEOMIC FINGERPRINTING OF HYDRAZINE TOXICITY IN PRIMARY RAT HEPATOCYTES.

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Any toxic chemicals will disturb gene expression and will give rise to a specific "fingerprint" of chemical toxicity. These changes in gene expression are extremely sensitive and unique, and can serve as biomarkers of the ultimate toxicity. Hydrazine (Hz) is used as fuel and propellant in aircraft and rockets. Hz is a hepatotoxic chemical, causing cirrhosis and necrosis in liver and it is a suspected carcinogen. There are limited data on the mechanisms of Hz toxicity, particularly on how Hz affects gene expression. To fingerprint Hz toxicity, rat primary hepatocytes were exposed to Hz at 0, 50 or 75 mM for 2 hrs at 37 °C. Total RNA was isolated at -2 (pretreatment), 0, 3, 6, 12 and 24 hrs post exposure to determine mRNA expression profiles using the Affymetrix RatTox U34 array. Cell lysates were also prepared at the same time points to determine global protein expression by 2D gel electrophoresis followed by peptide fingerprint and mass spectrometry. Our results indicated that (1) Hz modulated gene expression profiles, exhibiting time-course and dose-dependent responses for certain genes; (2) effects of Hz on gene expression profiles in rat primary hepatocytes were consistent with the results of cytotoxicity data, however, transcript responses seemed to be more sensitive; (3) based on approximate 110 proteins identified out of 1400 spots on 2D gels, protein expression was not well

correlated to the corresponding mRNA expression; (4) Hz modulation of transcripts resulted in time-specific patterns for certain genes, with an immediate significant induction of stress genes (ie., *hsp*40s and *hsp*70s), but a delayed dose-dependent up-regulation of apoptosis genes (ie., caspase-2 and fos) suggesting possible delayed apoptosis of cells. The transcriptomic and proteomic fingerprints of Hz toxicity in primary rat hepatocytes can help in identifying early biomarkers that can be used to predict human health effects.

56 IDENTIFICATION OF GENES ASSOCIATED WITH DRUG-INDUCED HEPATOCYTE PROLIFERATION IN THE RAT.

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Most rodent non-genotoxic hepatocarcinogens transiently increase hepatocyte proliferation during the first week of chronic exposure. Many of these agents also induce cytochrome P450 (CYP) enzymes at the level of gene expression by activating liver specific nuclear receptors such as the pregnane-X-receptor (PXR). Pregnenolone 16a-carbonitrile (PCN) and the glucocorticoid receptor (GR) agonist dexamethasone (Dex) are CYP3A inducers and PXR ligands. However, whereas PCN is a mitogen in the rat liver, Dex is not. The purpose of this study was to use Affymetrix transcript profiling technology to discriminate between gene expression changes caused by the two drugs and potentially identify genes associated with compound-induced hyperplasia. PCN (100mg/kg po), Dex (50mg/kg po) and corn oil (vehicle control) were administered daily to male Sprague-Dawley rats. RNA was isolated from rat livers after 6, 24 and 96 hours of drug exposure and used to generate cRNA probes for hybridisation to Affymetrix Rat U34A GeneChips. Genes were divided into functional categories and the expression changes (two fold change threshold and $P<0.05$ by Students t test) categorised according to whether they were common to both drugs or unique to one drug alone. CYP3A gene expression was upregulated at 24 and 96 hours by both drugs, although most changes in this category occurred at 6 hours and included genes involved in proliferation (cyclin G and CDK activating kinase). A small number of genes were uniquely regulated by PCN at 6 and 24 hours and mainly consisted of genes associated with proliferation (p21, cyclin B) and signalling (rhoB, IkB). Dex specific changes predominated at the later time points with suppression of genes involved in proliferative and immune pathways. A model is proposed in which PCN induces genes involved in cell cycle progression *via* PXR, whereas Dex suppresses DNA synthesis through the activation of GR, which has been previously demonstrated to inhibit growth factor induced signal transduction pathways.

57 ESSENTIALITY OF BRAIN EDEMA IN BINGE ETHANOL INDUCED BRAIN DAMAGE: PROTECTIVE EFFECT OF THE DIURETIC ACETAZOLAMIDE.

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Binge alcoholism results in brain damage. Though brain damage was suggested as a result of synaptic excitotoxic events, the underlying mechanisms are uncertain. Earlier results using the diuretic Furosemide (Faseb J. 12:221; 1998) suggest that non-synaptic regulation of brain cell volume may be more important for the occurrence of neuronal degeneration due to binge alcohol abuse than activation of synaptic excitatory receptors. As Furosemide is an efficient anti-oxidant agent, the protective effect of Furosemide was related to its anti-oxidant activity rather than reduction in edema (Hamelink et al., Soc. Neurosci. Abst. 2000). To test the essentiality of brain edema in binge ethanol induced brain damage, the diuretic drug acetazolamide (AZT) was used. AZT, a carbonic anhydrase inhibitor, is a different class of diuretic compared to Furosemide (Na/K/Cl channel inhibitor). AZT is not an anti-oxidant as established by the oxygen radical absorbance capacity (ORAC) assay. Male Wistar albino rats were separated into four treatment groups as control (saline), AZT (30 mg/kg b.w; i.p.), ethanol (ETOH; 30% orally gavaged), ETOH+AZT. Rats were treated for 8 days. Blood ethanol levels were comparable and insignificant in ethanol treated rats and ETOH+AZT treatment group. Brain edema was quantified following the method outlined by Elliot and Jasper (1949). AZT significantly ($p<0.001$) reduced brain edema in ETOH+AZT treated rats compared to ETOH treated rats. In addition to blocking brain hydration, AZT treatment reduced degeneration in ETOH intoxicated rats. The protective effect of the diuretic AZT was also confirmed in an *in vitro* model of binge ETOH toxicity using organotypic hippocampal-entorhinal cortex culture. The results of the *in vivo* study in tandem with *in vitro* organotypic culture suggest brain edema as an essential upstream event leading to brain damage independent of the anti-oxidant activity of the diuretic. Further studies aimed at quantitative analyses of brain edema in organotypic cultures are under way.

58 ROLE OF NITRIC OXIDE SYNTHASE-GENERATED REACTIVE INTERMEDIATES IN THE NEUROTOXICITY OF NITROARENES.

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We are using *E. coli*-expressed and purified neuronal nitric oxide synthase (nNOS) and the dinitrobenzene isomers (DNBs) as models of metabolism-dependent neurotoxicity. 1, 3-DNB is the most extensively studied of the DNB isomers, however the mechanism(s) involved in the CNS toxicity of 1, 3-DNB is unknown. The primary cellular targets involved in 1, 3-DNB-mediated CNS toxicity are glial cells and vascular tissue within discreet regions of the brain, including the cerebellum. Neuronal death appears to follow an initial damaging event to the glial and vascular elements and it has been postulated that 1, 3-DNB-mediated CNS toxicity is associated with the production of free radicals. Considering that nNOS is found in very high concentrations in the cerebellum, the same brain region targeted by 1, 3-DNB, we have initiated studies examining the interaction of the DNBs with nNOS. We have found that the DNBs redox cycle in the presence of nNOS resulting in an accelerated rate of NADPH oxidation in the rank order 1, 4-DNB > 1, 2-DNB > 1, 3-DNB. The increased NADPH oxidation leads to production of superoxide anion radical which traps the enzymatically-produced nitric oxide (NO•) resulting in increased nNOS activity. 1, 4-DNB is the most potent in this respect, with 1, 3-DNB being the least potent. However, although nNOS activity is increased by the DNBs, the products of the nNOS reaction, as measured by DCDHF oxidation, shift from NO• and L-citrulline in the absence of the DNBs, to the powerful oxidant peroxynitrite and L-citrulline in the presence of the DNBs. Furthermore, peroxynitrite production is blocked by inclusion of either superoxide dismutase or nitro-L-arginine in the enzyme assays. Collectively, these data demonstrate that in the presence of the DNBs, nNOS is converted from a NO• synthase to a peroxynitrite synthase. These studies have established a foundation from which more advanced experimentation into the production of reactive intermediates by the nNOS-DNB interaction can be based.(Supported by the Research Challenge Trust Fund at the University of Kentucky)

59 IMMUNOHISTOCHEMICAL AND *IN SITU* HYBRIDIZATION STUDY OF METALLOTHIONEIN (I & II, III) EXPRESSION IN THE BRAIN OF AGED DOG.

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Dogs are known to share a variety of morphological age-related brain changes with humans; the changes include neuronal loss and amyloidosis in the cerebral cortex, dystrophic axonal changes and ubiquitin deposition in the white matter. Iron also accumulates with age in the brain of dogs, the change may relate to the degenerative structural changes through iron-induced oxidative injury. Little is known, however, of the metallothionein (MT) related protection mechanism in the brain against oxygen radical species. To study the roles of MTs in the brain, the expression patterns of metallothioneins (I & II, III) were compared between the brains with severe age-related changes and those with mild changes (immunohistochemistry, *in situ* hybridazation). Total MT level was analyzed by the mercury-binding assay. Intense MT-I & -II immunoreactivity as well as signals for MT-I & -II mRNA were shown in the hypertrophic astrocytes in the brains with severe age-related changes. These MT-I & -II positive astrocytes dominated in the thalamus, cerebral cortex and medulla, where remarkable age-related changes were observed. In contrast, MT-I & -II expression was considerably weak in the brains of dogs with mild age-related changes. In aged dog brain, a population of hypertrophic astrocytes also showed MT-III immunoreactivity and signals for MT-III mRNA. On the other hand, neurons in both aged and young dog brains showed strong MT-III immunoreactivity and signals for MT-III mRNA regardless of the intensity of the age-related changes. Total MT concentration in brain was higher in gray matter than white matter and in young dogs than aged dogs: age-related loss of neurons, significantly rich in MT-III, is responsible for the latter finding. These results suggest, in addition to MT-I & -II, MT III may also be induced in astrocytes in relation to the progress of the age-related morphological changes, playing an important role in the protection of the brain tissue from the toxic insults responsible for the brain aging.

60 NEURONAL VESICULAR MONOAMINE TRANSPORTER (VMAT2) AS A NOVEL MARKER FOR METHAMPHETAMINE-INDUCED NEUROTOXICITY IN MICE.

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Methamphetamine (METH)is a synaptic toxicant in experimental animals. Neurochemical indices demonstrate loss of nerve terminal integrity in animals and humans following toxic doses of METH. Studies in human abusers with acute

METH intoxication report no decrement in postmortem measures of [3H]-dihydrotetrabenazene (DTBZ) binding to the neuronal vesicular monoamine transporter (VMAT2). This finding in humans, which is unexplored in mice, is unexpected in light of significant decrements observed in other neurochemical indices. In the present studies, [3H]-DTBZ binding was performed in striatal homogenates of METH-treated mice at time points between 1-85 days post-METH. These studies reveal that VMAT2 binding in striatal homogenate may not be a reliable index of nerve terminal loss at early time points (1-4 days) following METH intoxication. In contrast to significant loss of other neurochemical indices, [3H]-DTBZ homogenate binding gradually declined. On day 1 [3H]-DTBZ homogenate binding was not significantly decreased (-8%); by day 2, however, binding showed a significant decline (-34%), reaching a nadir by day 4 (-60%). To explore differences between [3H]-DTBZ binding and other measures of nerve terminal loss, [3H]-DTBZ homogenate binding was compared to [3H]-DA uptake into striatal vesicles 1 day post-METH. This measure of vesicular function was readily compromised, which is consistent with the early compromise of DAT(-77%)and TH (-65%). These results suggest that [3H]-DTBZ binding in homogenate may not adequately reflect nerve terminal status at early time points. However, at time points after day 4, loss of [3H]-DTBZ binding in homogenate (-60%) closely paralleled loss of TH activity (-51%). Over the course of 85 days, loss of [3H]-DTBZ binding and TH activity were not as substantial as loss of DAT binding and DA content. Recovery of measures occurred at the same rate; although measures demonstrating recovery persisted below control values, indicating a lasting neurotoxic effect in METH-treated mice.

61 THE TRIGGERING ROLE OF DOPAMINE IN NEUROTOXIC DAMAGE CAUSED BY AMPHETAMINE.

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Sponsor: S. Ali.

The breakdown products of dopamine (DA) are potentially neurotoxic but neuronal damage due to amphetamine (Amph) treatment may not only be produced by DA radical metabolites. The aim of present study was to explore the relationships between extracellular DA, glutamate (Glu) and taurine (Tau) and hydroxyl radical (OHž)generation during subchronic Amph treatment (5 mg/kg, 4 injections i.p. with 2-hour intervals). The extracellular levels of DA, Glu and Tau were estimated by means of HPLC and the generation of OHž with salicylate method in the rat neostriatum by microdialysis. Amph caused an immediate increase in the extracellular DA concentration up to 950%, which effect was quickly reduced to the baseline values. The subsequent Amph injections were followed by a much smaller increase in the extracellular DA concentration (about 300%). Amph produced marked increase in the OHž generation, the first wave was observed 80 min after the second injection and persisted during 2 hours (up to 700%), and the second increase incurred after the 4-th injection (up to 400%). Amph (5 mg/kg) caused a marked gradual increase in the Glu and Tau levels (up to 500 and 450% of the predrug value, respectively) by the end of experiment. Our results suggest a triggering role DA in neurochemical changes which lead to neuronal damage. The changes in extracellular DA, Glu, Tau and OHž reflect different subsequent phases of Amph neurotoxicity.

62 IDENTIFICATION OF A PROTECTIVE RESPONSE IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE.

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Alzheimer's disease (AD) is associated with increased levels of the toxic peptide, ß-amyloid (Aß). This disease is modeled in Tg2576 mice by overexpression of a mutant form of human amyloid precursor protein ($HuAPP_{Sw}$). However, despite high Aß levels, plaques do not develop in these mice until an average age of 12 months, and there is no neuronal loss in the hippocampus of mice as old as 16 months. Using an Affymetrix microarray system, we compared mRNA expression levels in the hippocampus and cerebellum of three six-month-old $HuAPP_{Sw}$ mice to three age-matched, non-transgenic controls. A ranking analysis based on Affymetrix's difference call was used to determine significant increases and decreases. RT-PCR of selected genes confirmed our results. Microarray analysis of the hippocampus demonstrated increased expression of transthyretin (TTR; 29.5-fold), a protein shown to sequester Aß and prevent plaque formation, as well as a number of genes involved in neuronal proliferation, differentiation, and tissue remodeling. Of the 23 genes increased in the hippocampus, five were involved in growth factor pathways. Three genes involved in the activation of the insulin-signaling pathway were differentially regulated, including a 16.6-fold increase in insulin-like growth factor 2 (IGF2). Immunohistochemistry confirmed the hippocampal increases of TTR and IGF2. The cerebellum is typically unaffected in AD and does not develop a significant age-dependent increase in Aß levels in $HuAPP_{Sw}$ mice. When comparing the cerebellar gene expression in $HuAPP_{Sw}$ mice to controls, the fold increase of TTR was reduced to 3.2-fold. Other genes differentially regulated in the hippocampus showed no changes at all in the cerebellum. Therefore, the slow progression and lack of full-fledged pathology in the hippocampus of $HuAPP_{Sw}$ mice may result from the increased expression of protective and growth-inducing genes. Specifically, TTR and the insulin-signaling pathway may play an important role in mediating this protective response. (Support: ES08089, ES10042, & BWF New Investigator Award).

63 OXIDATIVE DNA DAMAGE PRECEDES AMYLOID PLAQUE FORMATION IN AMYLOID PRECUSOR PROTEIN (APP) TRANSGENIC MICE: AN ANIMAL MODEL OF ALZHEIMER'S DISEASE.

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Reactive oxygen species (ROS) are implicated in neurodegenerative conditions such as Alzheimer's disease (AD), but their role in the mechanism of neurotoxicity is unclear. Here, we provide the first evidence that DNA oxidation precedes amyloid plaque formation in amyloid precursor protein (APP) mice, a transgenic mouse model of AD. To determine the relation of oxidative DNA damage, lipid peroxidation and amyloid beta (Aβ) plaque deposition, a characteristic hallmark of AD, we examined the frontal cortex, hippocampus and cerebellum of APP mice, compared to age-matched wild-type controls, for endogenous DNA oxidation evidenced by 8-oxo-2'-deoxyguanosine formation, and lipid peroxidation determined by thiobarbituric acid reactive substance formation. In the frontal cortex, oxidative DNA damage was enhanced within 6 weeks in APP mice, peaked at 5 months ($p<0.01$) and returned to basal levels by 7 months. DNA oxidation was similarly elevated in hippocampal but not cerebellar tissues. In contrast, plaque formation was not observed until after 3 months of age in the frontal cortex and hippocampus, nor was lipid peroxidation elevated in any tissue at any age. These results suggest that ROS-mediated oxidation of oligonucleotides, as distinct from lipid, may constitute an early event in the pathogenesis of AD. (Support: Canadian Institutes of Health Research)

64 POTENTIAL ROLE OF MITOCHONDRIAL COMPLEX II ACTIVITY IN REGIONAL DIFFERENTIAL CNS SENSITIVITY TO 1, 3-DINITROBENZENE-INDUCED ENCEPHALOPATHY.

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Exposure to 1, 3-dinitrobenzene (DNB) produces an edematous, glio-vascular lesion that is initially confined to brainstem nuclei with high energy requirements. Selective vulnerability of brainstem astrocytes to DNB is mediated by a ten-fold lower threshold for opening of the mitochondrial permeability transition (MPT) pore. Exposure of brainstem astrocytes to 100 uM DNB results in complete loss of mitochondrial membrane potential by 10 min, while their cortical counterparts remain unaffected for at least 1 hour. However, the loss of membrane potential in brainstem astrocytes is prevented by pretreatment with the known MPT pore inhibitor, cyclosporin A (CsA). DNB also decreases mitochondrial reducing potential by inhibiting FAD-linked reduction at complex II. Therefore, it is hypothesized that inhibition of mitochondrial complex II (succinate dehydrogenase) is causally linked to regional differences in susceptiblity to activation of the MPT by DNB. Using cultured neonatal rat brainstem and cortical astrocytes, complex II was localized histochemically. Complex II activity is significantly inhibited in both brainstem and cortical astrocytes at 0.5, 2, 5, and 24 hours following *in vitro* exposure to 100 uM DNB. Although the observed inhibition of SDH was prevented by pretreatment CsA in brainstem astrocytes after 0.5 and 2 hours, CsA pretreatment failed to significantly prevent inhibition of complex II activity in cortical astrocytes. These results suggest that either 1) inhibition of complex II is independent of the mechanism of DNB-induced opening of the MPT, or 2) differential regional regulation of the MPT subsequent to inhibition of complex II confers anatomical protection/vulnerability. However, the possibility that complex II activity is directly inhibited by DNB remains to be determined.

66 ANTIOXIDANT PROTECTION AGAINST ETHANOL-INDUCED CELL LOSS IN CEREBELLAR GRANULE CELL CULTURES.

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Ethanol exposure during development can cause decreases in neuronal cell populations resulting in permanent neurological deficiencies. Cerebellar granule cell cultures are vulnerable to ethanol toxicity and are useful for investigating mechanisms

of cell death. Oxidative damage resulting from ethanol-induced increases in reactive oxygen species, have been suggested as a mechanism of ethanol-induced cell death. Recent studies utilizing antioxidants showed protection against ethanol-induced cell loss. The purpose of the present study was to investigate the possible protective effects of the antioxidants, vitamin E and Trolox against ethanol-induced cell loss in cerebellar granule cell cultures during their peak time of ethanol vulnerability (one-day-in-vitro). Cerebellar granule cells were isolated from Long Evans rat pups and grown for 24 hours. Cultures were exposed to ethanol (0, 83, 166, 332mg/dl) for 24 hours and viable cells were quantified. In addition, cultures were exposed to 332mg/dl ethanol in the absence or presence of either vitamin E (0, 33, 100uM) or Trolox (0, 33, 66, 100uM) and assessed for viability. Results confirm a dose-dependent (14-29%) decrease in viable cell number following 24-hour ethanol exposure. Addition of antioxidants (vitamin E or Trolox) at concentrations of 100uM, protected against the ethanol-induced cell loss returning viability to that of controls (96% and 99.5%). This study suggests a role for oxidative stress, *via* either increased production of reactive oxygen species or decreased endogenous antioxidant levels, as a possible mechanism for ethanol-induced cell death in cerebellar granule cell cultures.

67 IDENTIFICATION OF A DRUG TARGETING EFFECT BY TERT-BUTYLHYDROQUINONE ON ANTIOXIDANT RESPONSE ELEMENT-DRIVEN GENE EXPRESSION USING MICROARRAYS.

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Increasing evidence indicates that the antioxidant response element (ARE) and Nrf2 mediate transcriptional activation of NADPH:quinone oxidoreductase and other detoxification enzymes in response to tert-butylhydroquinone (tBHQ). The current study was designed to investigate the gene expression profile induced by tBHQ and determine the time-dependent gene expression patterns associated with treatment. We have analyzed gene expression induced by tBHQ (10 mM) in IMR-32 human neuroblastoma cells by means of large-scale oligonuclotide microarray. Of the 9670 genes examined, 101 showed dynamic changes with treatment from 4h to 48h. The peak of transcriptional up-regulation happened at 8 hours and lasted until 24 hours. RT-PCR for selected genes also confirmed the similar gene expression pattern. Western Blot showed changes in the corresponding encoded proteins that remained elevated for 48 hours. Gene cluster (SOM) analysis was used to group these genes into 12 distinct clusters with striking patterns that appear to correlate with their specific function. These included genes involved in detoxification and cellular antioxidant defense, neuronal proliferation and differentiation, and signal transduction. A search for potential transcription factors binding site(s) in the 5'-flanking regions of the selected gene set was performed by MatInspector. The most notable observation was that genes induced by tBHQ had putative Nrf-2 binding sites, implying that a specific drug targeting effect exists. However, gene cluster analysis showed that not all of potential ARE-driven genes have the same expression profile indicating the complexity of trancriptional regulation is not dependent upon a single transcription factor or signal transduction pathway. (Funded by ES08089, ES10042, BWF New Investigator Award).

68 RETINOIC ACID INDUCED DISRUPTION OF CAMP-INDUCED HUMAN SVG CELL DIFFERENTIATION: MORHOLOGY AND GLOBAL GENE EXPRESSION EFFECTS.

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Previous work has shown that treatment of human SVG cells with 5 μM forskolin (F) and 200 μM 3-isobutyl-1-methylxanthine (iBMX) increases cAMP levels resulting in differentiation and dramatic morphologic changes. We show that co-treatment with 0.5 μM retinoic acid (RA) enhances these morphologic changes as measured by increases in the total number of processes ($p = 0.03$) and the length of processes contacting an adjacent cell (LPT; $p < 0.01$) over time. At 36h, RA inhibited regression of LPT ($p = 0.02$) with increases in mean process length ($p = 0.04$). A custom, SVG-specific cDNA microarray (2, 990 genes) was used to investigate the temporal changes in gene expression during cAMP induced differentiation. Temporal changes were identified using two different techniques. First, an incomplete block design, individual gene mixed-model ANOVA was used for analysis, followed by t-tests using a step-down Bonferroni adjustment. The mixed model included treatment, time, dye and treatment x time as fixed effects, and spots as random effects. To identify genes with significant expression changes during differentiation, least squares means of F/iBMX treated cells were compared to time-matched vehicle control samples utilizing t-tests. For comparison, a second technique utilizing Shannon Entropy filtering of normalized microarray data from treated, vehicle control, and untreated/time 0 h control cells, with Principal Components Analysis (PCA) of the filtered data to result in the identification of expression patterns was used. Results from the two divergent approaches were comparable and identified significant temporal and treatment effects on gene expression, although some differences were observed.

69 BREVETOXIN EXPOSURE AUGMENTS Ca^{2+} OSCILLATIONS IN CEREBROCORTICAL NEURONS.

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Florida red tide brevetoxins are potent sodium channel neurotoxins. Brevetoxins produce acute neuronal injury and death in cerebellar granule cells. This neurotoxic response is prevented by coapplication of tetrodotoxin or antagonists of the NMDA receptor. The neuroprotective efficacy of NMDA receptor antagonists has been attributed to the ability of brevetoxins to evoke the release of glutamate from cerebellar granule cells. Brevetoxins also produce a rapid increase in cytoplasmic $[Ca^{2+}]$ in cerebellar granule cells with a potency consonant with that for neurotoxicity. Brevetoxin produces Ca^{2+} influx through three pathways: NMDA receptors, L-type Ca^{2+} channels and the reverse mode of operation of the Na^+/Ca^{2+} exchanger. Oscillations in intracellular [Ca2+] induced by neuroreceptors in the plasma membrane provide critical signals to control cellular processes. To determine whether brevetoxin influence Ca^{2+} homeostasis generalized to neuron types besides cerebellar granule cells, we assessed the actions of brevetoxin (PbTx-2) on spontaneous Ca^{2+} oscillations in murine cerebrocortical neurons. This primary culture displays spontaneous oscillations in the presence of physiological concentrations of Mg^{2+}. PbTx-2 exposure produced a concentration dependent augmentation of the Ca^{2+} oscillation amplitude with a potency consonant with that for activation of sodium channels. The ability of brevetoxins to affect calcium signals may affect cellular processes involved in development and learning.

70 DEXTROMETHORPHAN PRODUCES CELL DEATH IN CEREBROCORTICAL NEURONS BY INHIBITION OF NMDA RECEPTORS.

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Dextromethorphan (DXM) is the active ingredient of commonly used antitussive preparations and represents a drug of abuse. We investigated the effect of persistent exposure of DXM and its metabolite dextrorphan (DX) on cell survival in murine primary cerebrocortical culture. A 48 h exposure to DX and DXM caused a concentration dependent rise in LDH efflux and number of pyknotic nuclei as visualized by Hoechst 33258 dye staining. Other NMDA receptor antagonists were also evaluated for their propensity to produce a neurotoxic action. The rank order of potency for neurotoxicity was dizocilipine ((+) MK-801) > phencyclidine (PCP) > DX > DXM. This rank order corresponds well with the respective binding affinities of these compounds for the NMDA receptor. Coexposure of cerebrocortical neurons with NMDA and noncompetitive antagonists reduced LDH efflux, further suggesting the involvement of NMDA receptors in the neurotoxicity. A 48h exposure to NMDA antagonists increased caspase-3 activity, but had no significant effect on caspase-1 activity. Intracellular Ca^{2+} concentrations have been found to be critical for cell survival especially during development. Real time alterations in intracellular Ca^{2+} were monitored in fluo-3 loaded cortical neurons exposed to MK-801 using a fluorescent plate reader (FLIPR). Acute exposure to MK-801 reduced the amplitude of calcium oscillations exhibited by neocortical neurons in a concentration dependent manner. These findings suggest that persistent exposure to DXM and DX produce toxicity in cortical neurons, which may be triggered by a decrease in intracellular Ca^{2+} due to the blockade of NMDA receptor.(Supported by DA 07218)

71 GENE TRANSFER OF THE Q/R EDITED GLUR2 GLUTAMATE RECEPTOR SUBUNIT PROTECTS MOTOR NEURONS FROM DISEASE-RELATED TOXICITY.

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There is considerable evidence that the presence of Ca^{++}-permeable AMPA receptors on motor neurons contributes to their vulnerability to excitotoxic injury and other insults associated with disease. Mutations in the SOD1 gene underlie some

familial cases of the motor neuron disease, amyotrophic lateral sclerosis (FALS). Using a primary culture model of FALS in which G93A mutant SOD1 was expressed in motor neurons of dissociated spinal cord cultures, we demonstrated that blockade of these receptors by the specific antagonist JSTX-3 prevented motor neuron death. In the present study, we tested the hypothesis that reducing Ca^{++}-permeability of AMPA receptors by overexpression of Q/R edited GluR2, the glutamate receptor subunit that prevents Ca^{++} entry through the receptor channel, would also be neuroprotective. Q/R-edited GluR2 (flop isoform) was cloned from rat cerebellar mRNA and expressed in motor neurons of mouse spinal cord cultures by intranuclear microinjection of plasmid expression vectors. Control vectors were "empty" vector and unedited GluR2 flop, which does not prevent Ca^{++} influx through the receptor channel, produced by site directed mutagenesis. Expression of GluR2 and localization to synapses was verified by double-label immunocytochemistry with anti-GluR2 and antibodies to other GluR subunits. 70-80% of motor neurons microinjected with G93A mutant SOD1 expression vector died over a 9 day period. Q/R edited GluR2 flop, but not unedited GluR2, significantly delayed motor neuron death induced by mutant SOD1. Thus, Ca^{++} influx during neurotransmission increases vulnerability of motor neurons to toxic insult and modulation of AMPA receptor properties is a potential therapeutic strategy in ALS. Supported by MDA.

72 CHARACTERIZATION OF HISTAMINE H1 RECEPTOR BINDING SITES IN FISH.

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The teleost brain is largely similar to that of higher vertebrates, but much less is known about specific neurochemistry. In mammals, the histamine receptor has been well characterized and consists of three subtypes, H1, H2, and H3. However, in fish, little work has been carried out in identifying the presence of different subtypes of histamine receptors or on pharmacological characterization of these types. The present study was designed to pharmacologically characterize the histamine H1 receptor in the fish brain using a radioligand binding assay. Specific [3H]pyrilamine binding increased in a linear fashion, with increasing concentrations of protein over the range of 50ug to 300ug. Association of [3H]pyrilamine to the membranes was time and temperature dependent, and equilibrium was achieved after 30 min. Saturation studies revealed that the specific binding of [3H]pyrilamine to the H1 receptor was saturable, reversible, and of high affinity. Competition studies indicated that the binding was readily displaced by specific histamine H1 agonists and antagonists. In contrast, the selective histamine H2 and H3 receptor agonists and antagonists were very weak competitors for the [3H]pyrilamine binding sites. These results present strong evidence for the existence of the histamine H1 receptor in fish brain, and that the pharmacological profile of the H1 receptor in fish brain is apparently similar to mammalian brain (Supported by NIEHS PP 1 P01 ES09563).

73 EVIDENCE OF HISTAMINE H1 AND H2 RECEPTORS IN FISH BRAIN AS CHARACTERIZED BY THE 2-DEOXYGLUCOSE METHOD.

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Non-mammalian species, such as fish, make ideal sentinel species for supplying essential information concerning human health issues related to environmental toxicology. However, methods for identification and characterization of mechanisms of neurotoxicity are underdeveloped. We have developed an *in vivo* neurochemical method to evaluate neuro-active agents, according to specific regional activities within the CNS by using [14C]2-deoxyglucose (2-DG) to trace regional metabolic activity in the fish brain (Choich et.al., 2001). We have used this method to characterize histaminergic pathways. Histamine is an amino acid neurotransmitter involved in arousal, attention, and sensory processing and cognition, and also plays a role in pituitary hormone secretion and appetite control. Structurally, the fish brain is well characterized, yet little information has been discovered about the existence and functioning of histamine neuropathways in fish. Living, healthy fish (4 fish, Tilapia nilatica, 10g-bw) were injected i.m. with 2uCi of [14C]2-DG and allowed to swim (30min.) in freshwater with and without a histamine H1 and H2 agonist (.01mM, 1mM, and 10mM of histamine dihydrochloride from Sigma). Fish were sacrificed and brains processed using autoradiography as described by Choich et.al. (Toxicology Methods, Nov. 2001). The experiment was repeated in triplicate. Using digital image analysis, a clear Dose-Response in regional 2-DG uptake was evident (increasing as dose concentration of histamine was increased, as compared to controls), providing evidence of histamine H1 and H2 receptors in fish, and demonstrating the applicability of our *in vivo* method to provide both quantitative and qualitative information on neuro-active agents and their direct effect on specific regions in fish brain. Supported by NIEHS PP 1 P01 ES09563.

74 *IN-VIVO* REACTIVITY OF THE AXONAL TOXIN 1, 2-DIACETYLBENZENE WITH NEUROFILAMENT AND MICROTUBULE PROTEINS.

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Repeated treatment of young adult Sprague-Dawley rats with 1, 2-diacetylbenzene (1, 2-DAB), but not with 1, 3-diacetylbenzene (1, 3-DAB), induces irritable behavior, reduced body weight gain and evolving leg weakness with neurofilament-filled giant axons in spinal cord and associated roots. 1, 2-DAB (but not 1, 3-DAB) reacts readily *in vitro* with amino acids (especially L-lysine and glycine) to form a blue pigment, which is also evident in the tissues and urine of systemically treated rats. In-vitro studies with purified bovine axonal proteins and rat spinal cord slices demonstrate reactivity of 1, 2-DAB (but not 1, 3-DAB) with neurofilament proteins relative to tubulin (unpublished data). Here, we report the action of 1, 2-DAB *vs.* 1, 3-DAB on neurofilament proteins and beta-tubulin in living nerve tissue. Surgically exposed male rat sciatic nerves were bilaterally bathed for 5 min. with saline + 2% acetone vehicle or vehicle containing 2, 5, 25 or 50 mM 1, 2-DAB or 1, 3-DAB. Treated nerves were excised, desheathed and homogenized in aqueous 8M urea containing protease-inhibitor cocktail. Protein (6 microgram) from each sample was subjected to Western blot analysis. Neurofilament triplet proteins, but not tubulin, showed a concentration-dependent decrease in nerve tissue treated with 1, 2-DAB (but not 1, 3-DAB); the hierarchy of reactivity (NF-H > NF-M > NF-L) with 25 mM 1, 2-DAB was comparable to that seen in spinal cord slices incubated *in vitro* with this chromogenic neurotoxin. These studies show that 1, 2-DAB preferentially targets protein components of axonal neurofilaments relative to that of microtubules. Toxicogenomic studies are underway to confirm these findings and to identify other axonal protein targets of 1, 2-DAB. Supported by NIEHS Center grants 1 P42 ES 10338 and 1 U19 ES11384.

75 GASOLINE OXYGENATES ENHANCE MUSCIMOL-STIMULATED CHLORIDE FLUX IN RAT BRAIN SYNAPTONEUROSOMES.

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Gasoline oxygenates are used to increase the efficiency of automobile fuel combustion. Commonly used additives are ethyl-*tert*-butyl ether (ETBE), methyl-*tert*-butyl ether (MTBE), and *tert*-amyl-methyl ether (TAME). The major metabolic product of ETBE and MTBE is *tert*-butyl alcohol (TBA), while TAME metabolism produces *tert*-amyl alcohol (TAA). These ethers and their alcohol metabolites have neurological effects similar to those of ethanol and anesthetics. One of the effects of ethanol is its interaction with the subtype-A of the receptor for the major inhibitory neurotransmitter, γ-aminobutyric acid (GABA). Since we had previously observed that these chemicals alter binding of $GABA_A$ receptor ligands, we studied their effects on the function of the receptor using rat-brain synaptoneurosomes (SNS), a preparation of sacs of pre- and postsynaptic membrane. $GABA_A$ receptor function was assessed by measuring the uptake of added $^{36}Cl^-$ into the SNS in the presence of varying concentrations of each additive or its metabolite, and muscimol, the agonist for the receptor. In addition, ethylether and ethanol were tested for the sake of comparison. $^{36}Cl^-$ uptake into the SNS was terminated after 5 seconds using picrotoxin, an agent that blocks the $GABA_A$ receptor channel. Each tested compound enhanced muscimol-stimulated uptake of ^{36}Cl at lower concentrations, while higher concentrations were less effective. The maximum stimulatory effect occurred at 10mM (for ethanol), 0.7 mM (for ethylether), 0.3mM (for MTBE), 1mM (for ETBE), 0.3 mM (for TAME), 5 mM (for TBA), and 3 mM (for TAA). The results suggest a potential role of the $GABA_A$ receptor in some of the neurotoxic effects of gasoline additives. (Supported by NSF grant IBN-981089)

76 EFFECTS OF ACRYLAMIDE AND 2, 5-HEXANEDIONE ON RAT TIBIAL NERVE SODIUM AND POTASSIUM CHANNELS.

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Changes in nodal Na and K channel quantity and distribution by acrylamide (ACR) or 2, 5-hexanedione (2, 5-HD) were evaluated as potential mechanisms in the production of neurotoxic symptoms. Sprague-Dawley rats (225-250g; n=3/group) were given either high (50mg/kg/d i.p. for 9 days) or low (2.8mM drinking water for 49d) dose ACR or high (400mg/kg/d for 39 days) dose 2, 5-HD; untreated littermates served as controls. At the endpoints, animals were ataxic and demonstrated significant hindlimb paralysis, particularly both ACR groups. Fixed tibial nerves were gently teased to single fibers and processed for identification of Na (monoclonal anti-sodium, clone K58/35, Sigma #S8809) and K (rabbit

anti-Kv1.1 voltage gated, Chemicon #AB5174) channels using anti-mouse IgG_1-FITC and TRITC-conjugated goat anti-rabbit IgG $F(ab')_2$ secondary antibodies, respectively. Fluorescence microscopy revealed control Na channels concentrated in a rectangular strip traversing the axon at each node. Control K channel fluorescence consisted of irregular-shaped paranodal strips of variable intensity. The following observations were made without knowledge of experimental group. ACR_{high} reduced nodal Na channel fluorescence with a narrowed and distorted distribution. ACR_{low} reduced Na channel fluorescence in the majority of nodes, although some retained normal shape and intensity. 2, 5-HD_{high} reduced fluorescence intensity of some; although many appeared normal. ACR_{low} and ACR_{high} produced a dramatic reduction in the intensity of K channel fluorescence; some nodes were devoid of fluorescence. 2, 5-HD_{high} produced a wide variety of results; fluorescence intensity ranging from normal to a complete absence of paranodal K fluorescence. In summary, ACR produced reductions in both Na and K channel concentration in nodes and paranodes independent of the dosing rate. 2, 5-HD affected K channels more than Na channels. Reduced Na and/or K channel quantity or altered distribution may precipitate failures in action potential propagation and neurobehavioral symptoms. Supported by the MCG Research Institute.

77 USING MICROELECTRODE ARRAYS TO IDENTIFY NEURAL RESPONSE PATTERNS FOLLOWING EXPOSURE TO A KNOWN NEUROTOXICANT.

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Exposure to non-lethal amounts of neurotoxic agents disrupts brain activity, and often results in negative neurobehavioral consequences. To develop effective treatments for such exposures, it is important to understand how the neurotoxic compounds alter neuronal activity and to correlate these changes with the behavior deficits. There are a variety of methods that can be utilized for assessing altered neural communication, one of which is *in vitro* work with microelectrode arrays. Slices from specific brain regions, selected for their known contributions to behavior, are used to assess spontaneous electrical activity in the tissue. Modulation of spontaneous activity by different doses of neuroactive compounds is also assessed. Trimethylolpropane phosphate (TMPP) is a known neuroactive toxicant formed during partial pyrolysis of some turbine lubricants. TMPP has convulsant properties, and causes lasting, likely permanent, changes in neurobehavioral responding following short term exposure to low doses. The goal of this research was to evaluate the changes in responsivity of neural systems that could account for the previously observed *in vivo* effects. Rat hippocampal slices were mounted on 64-channel microelectrode arrays (Panasonic Med. System) and evoked field potentials were recorded. Tissue exposure to TMPP produced dramatic changes in the evoked potentials characterized by a shorter latency to peak response, and an erratic wave form. Similar to the lasting behavioral effects, TMPP exposure caused lasting changes in responses of neural circuits in hippocampal slices. Extensive washing of the preparation did not alleviate the altered evoked potentials. The results suggest that the convulsant properties of TMPP are sufficient to cause long term changes in neural responses that may account for the lasting neurobehavioral consequences. The results also support the application of microelectrode array techniques for evaluation of neurotoxic effects.

78 RECRUITMENT OF PAROXYSMAL AFTER-DISCHARGE IN A MOUSE BRAIN AMYGDALAR TISSUE SLICE USING A PLANAR MICROELECTRODE ARRAY.

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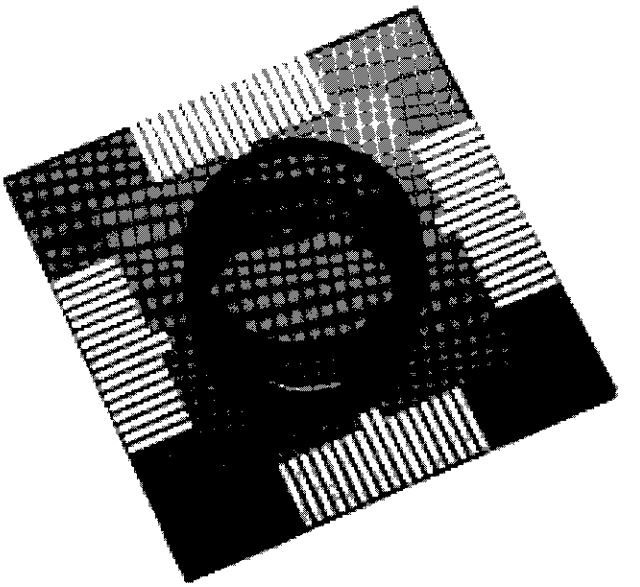
The Panasonic Med.-64 microelectrode array system (Panasonic, Japan) was utilized to successfully demonstrate recruitment of paroxysmal after-discharge responses of *in vitro* tissue slice preparations from adult mice. Slices were prepared to allow placement of both the amygdala and pyriform cortex on a single Med.-64 microarray probe. Repeated tetanic pulse trains [intensity = 400 microamps; pulse train duration = 20 ms; pulse duration = 2 ms; interpulse interval = 0.5 ms] were delivered to a single electrode within the basolateral amygdala. A second electrode position demonstrating a consistent and reliable evoked response to the amygdalar stimulation was selected to measure recruitment responses. With successive stimulation of the basolateral amygdala during a single 2-h session, a paroxysmal spike-wave discharge (SWD) response significantly exceeding the amplitude of the initial evoked response was observed to develop at the pyriform cortex recording site in 75%+ of all slices tested. It was demonstrated that an *in vitro* murine tissue slice preparation could be used to demonstrate electrically induced recruitment of paroxysmal after-discharge responses in a remote brain area. This system may provide a novel clinical tool for investigation of mechanisms of epiletogenesis, as well for evaluation of toxicant-induced changes in neural function and antiepileptic drug (AED) treatments.



79 SPATIOTEMPORAL PATTERN OF NEURODEGENERATION IN CNS OF ACRYLAMIDE (ACR)INTOXICATED RATS.

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The influence of dose-rate on spatiotemporal characteristics of neurodegeneration in CNS of ACR intoxicated rats has not been well documented. Therefore, animals were exposed to daily ACR at either 50 mg/kg (i.p.) or 21 mg/kg (p.o.) and brains and spinal cords were processed using de Olmos' amino cupric silver stain for detecting degenerating nerve cell bodies, dendrites, axons and terminals. For each dose-rate, experimental times were selected according to the development of ACR-induced neurological defects; i.e., ataxia and hindlimb foot-splay. Results show that, in spinal cord, ACR intoxication at either dose-rate produced nerve terminal degeneration in several gray matter regions (e.g., layers 5-7 of intermediate zone, lamina 10, intermediolateral cell column). However, axon degeneration in spinal white matter was related to dose-rate; i.e., the lower ACR dose rate (21 mg/kg/d)was associated with significant axon degeneration primarily in the gracile fasciculus and pyramidal tract, whereas during higher dose-rate exposure axon involvement was rare. In cerebellum, selective Purkinje cell damage was evident regardless of dose-rate. This effect was characterized by early degeneration of Purkinje cell dendrites in the molecular layer and nerve terminals in cerebellar and vestibular nuclei. The onset of axon argyrophilia in cerebellar white matter was relatively delayed. In hindbrain, both dose-rates produced predominant nerve terminal degeneration in precerebellar nuclei (e.g., lateral reticular nucleus), gracile and cuneate nuclei and certain nuclei of the reticular formation (e.g., pontine nucleus). Neither dose-rate caused axon degeneration in hindbrain white matter. Thus, nerve terminal degeneration in the CNS is a consistent effect of ACR across dose-rates, whereas axon degeneration is either delayed or occurs only during low dose intoxication. This supports the hypothesis that the nerve terminal is a primary site of ACR action. Sponsored by NIH grant ES03830-16

80 ACRYLAMIDE (ACR) BINDS SPECIFIC PRESYNAPTIC PROTEINS IN RAT BRAIN.

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We hypothesize that the nerve terminal is the primary site of ACR action and that binding of this toxicant to critical thiol-containing SNARE proteins disrupts presynaptic structure and function. As an initial investigation of molecular mechanisms, we characterized the binding of ACR to synaptosomal proteins. Rat cerebral cortex synaptosomes (250 µg protein) were prepared on Percoll gradients and were incubated in oxygenated medium for 1 hr with ^{14}C-ACR (7 µCi; 5 mCi/mmole). Labeled synaptosomal proteins were resolved by gel electrophoresis and then trans-

ferred to nitrocellulose membranes. Protein bound radiolabel was visualized on autoradiographs formed by exposing membranes to X-ray film for 21 days. Comparion of coomassie blue-stained gels with autoradiographs revealed that, among numberous potential targets, six ACR-labeled proteins could be identified. The estimated molecular weights of several unknowns matched that of specific SNARE proteins; e.g., SNAP-25 (25 kDa), synaptophysin (38 kDa) and synaptotagmin (58 kDa). Radiolabeling of proteins could be completely prevented by preincubation with the sulfhydryl reagent N-ethylmaleimide (NEM; 4 mM), but not by exposure to propionamide (10mM) or to the monothiol-reducing agent, dithiothreitol (4 mM). Determination of the relative sulfhydryl reactivates of ACR, NEM and idoacetamide (IA) showed that these chemicals were equally efficacious with respect to reducing DTNB-determined synaptosomal -SH content, although the order of potency differed; NEM>IA>ACR. These data suggest that ACR forms sulfhydryl adducts with specific presynaptic proteins and provide initial support for our hypothesis that ACR neurotoxicity is mediated by nerve terminal sites of action. Sponsored by NIH grant ES03830-16.

81 ELECTRON MICROSCOPIC EVALUATION OF THE CEREBELLAR CORTEX IN ACRYLAMIDE EXPOSED RATS.

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Male Sprague-Dawley rats (250-275 g) were administered 50 mg/kg acrylamide intraperitoneally for 5 or 11 days, and then sacrificed for ultrastructural examination of the cerebellar cortex. Light microscopy revealed regions of vacuolization of the molecular layer in treated rats. Beginning on day 5, transmission electron microscopic study of the cortex demonstrated a selective effect on the Purkinje cells. Some nuclei were eccentric in location. Their perikaryal cytoplasm contained disordered and diminished Nissl substance, particularly its rough endoplasmic reticulum component, and increase in tubular and vesicular profiles. Occasional large double membrane lined vacuoles, likely representing swollen astrocytic intrusions into the altered perikaryon, were noted. Swollen Purkinje cell dendrites containing electron dense aggregates of degraded organelles were present in the molecular layer. Altered mitochondria were a prominent feature of these degraded masses, which sometimes filled post-synaptic regions of the dendrites. Adjacent astrocytes were swollen. Rare degenerating myelinated axons were seen in the granular layer. These findings indicate that the Purkinje cell is a cerebellar target during acrylamide intoxication, and confirm in part the findings of an earlier electron microscopic study (Cavanagh and Gysbers, J. Neurocytol. 12:413-437, 1983). We also demonstrate a cellular and ultrastructural basis for our companion silver impregnation study of degenerating cerebellar Purkinje cells (Ross et al., Abstracts of the 2002 Society of Toxicology meeting). Supported by NIH grant ES03830-16.

82 *N, N*-DIETHYLDITHIOCARBAMATE, BUT NOT *S*-METHYL- *N, N*-DIETHYLDITHIOCARBAMATE, PRODUCES A DISULFIRAM-LIKE SCHWANNOPATHY IN RATS.

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Disulfiram, a dithiocarbamate drug used in alcohol aversion therapy, produces a peripheral neuropathy that was characterized in rats as a segmental demyelination accompanied by generation of *S*-(*N, N*-diethylcarbamoyl)cysteine (DETC-Cys) adducts. To assess the role of cysteine carbamylation in disulfiram toxicity, 2 disulfiram metabolites, *N, N*-diethyldithiocarbamate (DEDC) and *S*-methyl- *N, N*-diethylthiocarbamate (MeDETC), were administered parenterally to male Sprague-Dawley rats *via* osmotic infusion pumps for 4 and 8 weeks. Following exposure, hemoglobin and spinal cord axonal proteins were isolated and analyzed for the presence of DETC-Cys adducts, while peripheral nerve and spinal cord sections were obtained and examined by light and electron microscopy for the presence of morphological lesions. Globin preparations from both DEDC- and MeDETC-exposed animals were found by RP-HPLC to contain a late-eluting β-globin peak, the quantity of which increased as a function of exposure level and duration, consistent with the formation of a DETC-Cys adduct on the β_3-globin chain. The presence of DETC-Cys protein adducts was verified after hydrolysis of both globin and spinal cord axonal proteins by LC-MS/MS analysis. The quantity of DETC-Cys adducts produced on hemoglobin and axonal proteins was two fold greater for MeDETC relative to DEDC. Demyelinated axons, myelin edema, and other Schwann cell lesions similar to those seen with disulfiram treatment, were observed in all peripheral nerves examined from animals treated with 0.25 or 0.35 mmol/kg/d DEDC for 8 weeks. No lesions were detected following exposure to MeDETC at any time point or dose level. These results indicate that parenteral administration of DEDC produces a neuropathy similar to disulfiram, but the lack of peripheral nerve lesions following MeDETC treatment in the presence of higher levels of DETC-Cys adducts suggests that formation of DETC-Cys adducts is not responsible for development of neuropathy. (Supported by NIEHS grants ES06387 & ES00267).

83 PROTECTION AGAINST METHANOL-INDUCED RETINAL TOXICITY BY LED PHOTOSTIMULATION.

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Decrements in mitochondrial function have been postulated to be involved in the pathogenesis of macular degeneration, diabetic retinopathy, Leber's hereditary optic neuropathy and methanol intoxication. Formic acid is the toxic metabolite responsible for the retinal and optic nerve toxicity produced in methanol intoxication. Previous studies in our laboratory have documented formate-induced mitochondrial dysfunction and retinal toxicity in a rodent model of methanol intoxication. Photostimulation by monochromatic red to near-infrared radiation has been postulated to enhance mitochondrial activity by stimulation of cytochrome oxidase. The present studies were undertaken to test the hypothesis that exposure to 670 nm light from light-emitting diode (LED) arrays would protect the retina against the mitotoxic and cytotoxic actions of formate. One group of animals was intoxicated with methanol in the absence of LED treatment and a second group was intoxicated with methanol and exposed to LED treatment (670 nm for 1 min 45 seconds = 50 mW/cm^2, 4 joules/ cm^2) at 5, 25, and 50 hours after the initial dose of methanol. Blood formate concentrations did not differ between the two treatment groups and averaged 5.6 ± 1.0 mM at 72 hours. At 72 hours of methanol intoxication, retinal function was assessed by measurement of ERG responses and retinas were prepared for biochemical and histologic analysis. ERG responses recorded in methanol-intoxicated animals revealed profound attenuation of both rod-dominated and UV-cone mediated responses. In contrast, methanol-intoxicated animals exposed to LED treatment exhibited a complete recovery of rod-dominated ERG responses and partial recovery of UV-cone mediated ERG responses. These data provide evidence that LED phototherapy protects the retina against the cytotoxic actions of formate and are consistent with the hypothesis that LED photostimulation improves mitochondrial respiratory chain function. (Supported by NIH RO1-ES06648, RO1-EY11396, P30-EY01931 and NASA NAS8-99015).

84 THE INDUCTION OF AMYLOID PRECURSOR PROTEIN AND α-SYNUCLEIN IN ASTROGLIA BY DIETHYLDITHIOCARBAMATE (DDC) AND COPPER WITH OR WITHOUT GLUTATHIONE (GSH).

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α-synuclein has been hypothesized to provide a nucleation centre during the formation of senile plaques. It has been shown that α-synuclein undergoes self-oligomerization when β-amyloid (Aβ) is present. Glial cultures produce the Kunitz-protease inhibitor (KPI) -containing APP. The KPI-containing APP protein may be implicated in Alzheimer's disease (AD) pathogenesis. Astroglia may play a role in the pathogenesis of AD. The role of xenobiotics in the pathogenesis of neurodegenerative disease has been suggested. The mechanism of DDC neurotoxicity is thought to involve Cu++ chelation and to interact with SH group to destroy the GSH homeostasis and inhibit sulfhydryl enzymes. GSH has been shown to protect against DDC cytotoxicity. Cu has been shown to induce the self-oligomerization of α-synuclein. APP contains a Cu-binding site. APP reduces bound Cu++ to Cu+, and could induce the formation of hydroxyl radicals. In this study, rat astroglia were grown in complete medium on glass chamber slides or T75 flasks. Astroglia were treated with DDC 17.5 μg/ml, Cu 0.2 μM, or DDC 17.5 μg/ml + Cu 0.2 μM in medium for 1 hr. Cells were washed in PBS and refed with complete medium with or without 10 mM GSH. Immunocytochemistry was performed at 4 and 8 hr post treatment using APP or α-synuclein primary antibodies followed by gold conjugated secondary antibody. Results showed the increase of APP and α-synuclein production occurring in a time-dependent manner. At 4 hr post treatment, the cells contained small positively stained material deposited throughout the cytosol. This material increased in size and density over time, and by 8 hr it appeared as a dense aggregate surrounding the nucleus. Western Blots were performed at 4 and 8 hr post treatment using APP or α-synuclein primary antibodies followed by horseradish peroxidase-linked secondary antibody. Immunoblots supported immunocytochemistry results. GSH showed significant protection at 8 hr post treatment.

84A CYCLIC AMP-DEPENDENT PROTEIN KINASE INDUCTION BY POLYCHLORINATED BIPHENYLS (PCBs) STIMULATES CREB PHOSPHORYLATION *VIA* A CALCIUM-DEPENDENT, PKC-INDEPENDENT PATHWAY IN CORTICAL NEURONS.

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We have previously demonstrated that the PCB mixture, Aroclor 1254 (A1254), increases the phosphorylated form of CREB (pCREB), the cAMP-responsive element binding protein. This transcription factor is important in nervous system development and plasticity. Phosphorylation of CREB results in its activation and is

mediated by a variety of stimuli, including activation of protein kinases A and C and increases in intracellular calcium. As such, the present experiments were designed to determine the mechanism(s) by which PCBs increase pCREB. Using digital microscopy, semi-quantitative fluorescent immunocytochemistry of serine-133 phosphorylated CREB was performed in primary cultures of cortical neurons in the presence and absence of A1254 and agonists and antagonists of PKA, PKC, and intracellular Ca^{2+}. Cellular pCREB flourescence intensity was analyzed with the aid of image analysis software. The pCREB responses were represented as Gaussian populations where the half-maximal fluorescence (EC_{50}) of the population was the comparator across treatment groups. In controls, pCREB was constitutively and non-uniformly expressed. A1254 (2-20 μM) increased pCREB immunoreactivity in a dose-dependent manner. A1254 (10 μM) or the agonists glutamate, forskolin and phorbol myristate acetate increased the EC_{50} values for pCREB fluorescence by 36±5, 51±15, 47±9 and 40±10%, respectively. PKA inhibitor peptide (30 min preincubation) completely inhibited, whereas BAPTA-AM or tetrodotoxin partially inhibited, and the PKC inhibitor, bisindoylmaleimide, did not inhibit pCREB induction by A1254 (10 μM, 40 min). We also examined whether cAMP is involved in the activation of PKA by PCBs. The adenylate cylcase activator, forskolin, but not A1254 (2-20 μM) elevated intracellular cAMP levels. These data support a role of a PKA and intracellular Ca^{2+} pathways involved in PCB-induced CREB phosphorylation. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy).

84B ACTIVATION OF MAP KINASE BY THE PERSISTENT BIOACCUMULATIVE TOXICANTS PCBs AND PBDEs IN DEVELOPING CORTICAL CULTURES.

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Alteration of calcium signaling has been proposed as a possible mechanism contributing to the developmental neurotoxicity of polychlorinated biphenyls (PCBs). Little is known, however, regarding the actions of structurally related compounds such as polybrominated diphenyl ethers (PBDEs), flame retardants whose levels are rising rapidly in the environment. We examined the actions of the PCB mixture Aroclor 1254 (A1254), which is known to alter calcium homeostasis, and a PBDE mixture (DE71), on activation of the mitogen-activated protein kinase (MAPK) cascade. MAPK is a critical regulator of cell division and differentiation which can be activated by numerous signals including increases in intracellular calcium. Immature primary cultures of rat cortical neurons were deprived of serum overnight and exposed to A1254, DE71, or the agonists glutamate and KCl. Activation of MAPK was assessed by measuring the dually-phosphorylated, active form of MAPK by SDS-PAGE and immunoblotting with a phospho-specific antibody. In immature cortical neurons, activation of ionotropic and metabotropic glutamate receptors with glutamate resulted in a time- and concentration-dependent increase in phospho-MAPK. This activation could be partially blocked using calcium-free buffer in the presence of the calcium chelator BAPTA. Similar effects on MAPK activation were observed after depolarization of cortical neurons with KCl. Stimulation with both A1254 and DE71 (1-30 μM) resulted in a time- and concentration-dependent increase in phospho-MAPK. A1254 was more potent than DE71, and the effects were partially blocked in the absence of calcium. These results suggest that PCBs and the structurally related PBDEs can affect the MAPK kinase cascade, a critical mediator of cell development and neuronal plasticity. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy).

85 QUANTIFICATION OF CYP GENE EXPRESSION DETERMINED BY LIGHTCYCLER TECHNOLOGY.

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Changes in drug-metabolizing enzymes sometimes alter toxicological consequences. Although drug-metabolizing enzymes, especially cytochrome P450 (CYP) levels, after exposure of chemicals are generally assessed by expressing protein levels using western blotting, it is considered that quantification of their gene expression levels provides equivalent or more valuable information. However, the combined method of RT-PCR and Northern blotting, which has been generally used, is time consuming. We have achieved quantification of CYP gene expression levels by real-time PCR (LightCycler™, Roche). RNA was isolated from rat liver and reverse transcription was conducted. Gene expression of CYP was quantified by the real-time PCR method consisting of PCR in a capillary tube and online monitoring of fluorescence of SYBR Green I, which is specifically bound to stranded DNA produced by PCR reaction. Using this method CYP induction after administration of dexamethasone (DEX) and 3-methylchoranthrene (3-MC), well known enzyme inducers, were examined. DEX (60 mg/kg) or 3-MC (30 mg/kg) was given by an i.p. injection to female SD rats, and liver samples were collected 24 hours later. Primers corresponding to CYP molecular species (CYP1A2, 2B1, 2B2, 3A2, 3A9, 3A23) and ß-actin were used and all the molecular species examined were quantifiable. Significant enzyme induction was observed in CYP2B1 and 3A23 in the DEX treated group and in CYP 1A2 in the 3-MC treated group. The advantages of this method are that analysis time can be reduced to less than 60 minutes without risk of contamination and use of labor-intensive gel electrophoresis, and that multiple samples (maximally 32 samples containing controls) can be examined simultaneously. These results suggest that real-time PCR by LightCycler™ can be a useful tool for rapid determination of altered CYP gene expression and possibly other target genes induced by chemical exposure.

86 EFFECT OF PCB EXPOSURES DURING DEVELOPMENT ON THE P450-MEDIATED METABOLISM OF ORGANOPHOSPHORUS INSECTICIDES IN RAT LIVER MICROSOMES.

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Phosphorothionate insecticides can be activated to their oxon metabolites by desulfuration or detoxified by dearylation, both reactions are mediated by P450's. The effect of gestational/lactational exposure to PCB's (polychlorinated biphenyls) on hepatic microsomal metabolism of the organophosphorus insecticides diazinon, azinphosmethyl, dimethoate, and coumaphos was determined. The dams were administered the PCB mixture Aroclor 1254 from GD6 to PND 22. The potential of detoxication, *via* dearylation, of coumaphos and diazinon was monitored using HPLC methodology in naive male rats (PND 3, 12, 21, 33, 70), and in male and female rats exposed to PCB's (PND 15 and 23). Data showed a general increase in pyrimidinol (diazinon dearylation) production with age, with 70d and 33d statistically different from one another, and from the other ages 3, 12, 21 which were not statistically different from one another. PCB treated rats showed a statistically significant increase in pyrimidinol production at all ages and in both sexes. Analysis of coumaphos phenol production showed a general age-related increase with no statistical difference within the naive rat group; however data in PCB treated animals showed a similar trend as with diazinon dearylation. Diazinon, dimethoate, and coumaphos desulfuration potentials (measured indirectly using acetylcholinesterase inhibition) showed no significant differences among ages, but did exhibit a general age-related increase in naive rats. Data for azinphosmethyl activation exhibited an age-related increase with 70d and 33d not statistically different from one another, but different for the rest of the ages studied. PCB treated rats showed significantly greater desulfuration for diazinon, azinphosmethyl, and coumaphos at all ages and with both sexes. Dimethoate desulfuration showed no statistical difference in the PCB treated groups. The data indicate that age-related differences in desulfuration/dearylation potential for naive rats is compound specific as is the ability to be induced by PCB exposure (EPA R 825296-01).

87 *IN VITRO* METABOLISM OF OCTAMETHYLCYCLOTETRASILOXANE BY HUMAN AND RAT MICROSOMES.

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Exposure of rats to Octamethylcyclotetrasiloxane (D4) induces CYP450 enzymes in the liver, and causes hepatomegaly and transient increases in hepatocyte proliferation similar to phenobarbital (PB). It also appears that these enzymes are capable of recognizing D4 as a substrate and may play a role in its metabolism and elimination in the rat. This study was designed to determine if D4 is metabolized by liver microsomes from humans or rats and to identify which specific CYP450(s) are responsible for the metabolism in humans. 14C-D4 was incubated with recombinant (r) human CYP450s and liver microsomes (rat & human) at 37°C for up to 2 hr and at various protein concentrations. Incubation extracts were analyzed by HPLC with radiometric detection. Chromatograms from PB-rat incubations contained at least 8 distinct peaks (metabolites M1-8) whereas only 2 metabolites (M5, M8) were detected in saline-rat incubations. Human incubations gave rise to predominately 1 metabolite (M8) with M5 detected only at high protein concentrations. Of the rCYP450s evaluated, only rCYP2B6 (M5 & M8) and rCYP3A4 (M8) produced metabolites. Formation of M8 and/or M5 by human microsomes and rCYP3A4 was not proportional to time or protein concentration. Formation of M8 by rCYP2B6 was proportional to protein concentration but not time. M8 formation in saline-rat incubations was proportional to protein concentration but not time. In PB-rat incubations a few metabolites showed proportionality between formation and incubation time and/or protein concentration, however M8 did not. These experiments revealed that rat and human liver microsomes are capable of me-

tabolizing D4 and PB induction in rats greatly enhances the ability of liver microsomes to metabolize D4 to a variety of metabolites. The use of rCYP450s and inhibitory polyclonal antibodies demonstrated that human CYP2B6 and CYP3A4 are likely involved in the observed metabolism. Supported in part by Silicones Environmental, Health and Safety Council of America

88 PRECISION-CUT HUMAN LIVER SLICES AS A MODEL TO EXAMINE CHANGES IN CYTOCHROME P450 EXPRESSION TO PREDICT OR PRECLUDE POTENTIAL DRUG-DRUG INTERACTIONS.

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Precision-cut human liver slices offers a practical alternative to hepatocyte cell cultures to examine cytochrome P450 (CYP) induction by compounds to predict or preclude potential drug-drug interactions. Induction of P450 gene expression was measured in human liver slices by real-time PCR using probes/primers developed to specifically recognize CYP1A1, CYP1A2, CYP3A4, CYP2C8, CYP2C9, or CYP2C18 mRNA. Liver slice viability was monitored by measurement of ATP, GSH, and K^+ content. Minimal loss in slice viability occurred between 24 and 48 h (1.2- and 1.7-fold decreases in K^+ and ATP content, respectively), whereas, more significant losses of viability occurred by 72 h. CYP1A1 and CYP1A2 gene expression was induced by β-napthoflavone (50 μM), with respect to vehicle control, 99- and 110-fold at 24 h of treatment and 19- and 55-fold at 48 h, respectively, which indicated that maximal induction occurred at or prior to 24 h. Inducers of CYP3A4, carbamazepine (CARB, 50 μM), rifampicin (RIF, 50 μM), phenobarbital (PB, 0.5 mM), and dexamethasone (DEX, 50 μM) induced CYP3A4 gene expression 2.4-, 4.6-, 7.6- and 3.6-fold at 24 h, and 2.9-, 7.1-, 18-, and 10-fold at 48 h, respectively. Known inducers of CYP2C enzymes, RIF, PB, and DEX increased CYP2C9 mRNA expression by 1.9-, 3.1-, and 2.3-fold at 24 h and by 1.9-, 3.7-, and 3.9-fold at 48 h, respectively. RIF, PB, and DEX induced CYP2C8 mRNA expression by 6.0-, 7.0-, and 7.4-fold at 24 h and 2.7-, 5.7-, and 4.6-fold at 48 h, respectively. DEX induced CYP2C18 mRNA by 48 h (2.4-fold), whereas PB and RIF had little effect. The levels of induction of CYP2C and CYP3A4 mRNA were similar to induction levels found recently in human hepatocytes. These studies indicated that induction of P450 gene expression can be measured in human liver slices with a single treatment of inducer within 24 h of slice culturing and up to 48 h with minimal reduction in slice viability.

89 DEVELOPING AN *IN VITRO* MODEL SYSTEM TO EVALUATE SWITCH-LIKE BEHAVIORS OF HEPATOCYTES IN RESPONSE TO VARIOUS ENZYME INDUCERS.

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Cytochrome P450 induction in liver by a variety of enzyme inducing chemicals demonstrates switch-like behaviors. As dose increases, more cells become fully induced rather than observing a proportionate induction in all cells in the liver. These inducers interact *via* different receptor systems, indicating that this switch-like response is a generic property of induction with these cells. The molecular mechanisms that control these switches remain unknown. We have developed an *in vitro* model system using cultured rat hepatocytes to study this phenomenon with prototypical inducers. In this study we examined induction of CYP 1A1 protein and CYP 1A1 mRNA by PCB 126, a prototypical aryl hydrocarbon receptor (AhR) agonist. Hepatocytes from male Sprague-Dawley rats were treated for 24 hours after attachment on plates at PCB126 concentrations ranging from 10^{-5} to 10^{-10} M. Total CYP1A1 protein was evaluated in cell homogenates by densitometry of Western blots and for CYP1A1 mRNA by quantitative PCR. The EC50s for induction of both protein and mRNA were in the range of 2 to 5 nM. These integrated responses of populations of hepatocytes were compared with responses of individual cells by visualization of treated cells on plates. Protein was visualized by CYP 1A1 immunohistochemistry. While induction of individual cells and the Dose-Response for increasing numbers of cells stained with increasing dose was evident by immunohistochemistry, further refinements of imaging techniques are necessary to derive quantitative estimates of numbers of cells induced at each dose level. Nonetheless, the combined evaluation of population and cell level responses of hepatocytes offered by this *in vitro* model permits characterization of the induction switch and provides a system to probe the molecular mechanisms that underlie non-linear dose-response behaviors for these receptor-mediated toxicants. This work was supported by a contract from the American Chemistry Council.

90 EVALUATION OF AFLATOXIN B1 AND OTHER CHEMICALS IN GENTEST© CELL LINES: CYP-MEDIATED TOXICITY IS DIMINISHED BY CO-TREATMENT WITH SPECIFIC INHIBITORS.

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Many xenobiotics are more toxic when metabolized by cytochrome P450 enzymes (CYPs). The intent of this study was to test such chemicals in Gentest© cell lines, human lymphoblasts that have undergone stable transfections resulting in expression of human CYPs. Aflatoxin B1 (AB1) was tested in the cell lines h1A2v2, h3A4/OR, h2E1/OR, 2C9-Arg/OR, 2D6-Val, MCL-5 (CYP1A2, 3A4, 2E1, 2A6, and elevated native 1A1), and the control lines cHol and cHyg (no CYP over-expression) for 72 hours alone and concurrently with inhibitors. Inhibitors used were as follows: 5 μM furafylline (1A2), 10 μM ketoconazole (3A4), 10 μM sulfaphenazole (2C9), 10 μM 4-methylpyrazole (2E1), 10 μM quinidine (2D6), and 15 μM aminobenzotriazole (ABT, all). Furafylline inhibited CYP-mediated AB1 toxicity completely in the h1A2v2 cells but not in the MCL-5 cells. Ketoconazole inhibited AB1 toxicity completely in the h3A4/OR cells but not in the MCL-5 cells. Co-treatment with ABT in the MCL-5, h1A2v2, and h3A4/OR cell lines did not inhibit toxicity. AB1 did not demonstrate toxicity in the other cell lines. Aflatoxin B2, B2a, and G2a were not toxic to any of the cell lines at the concentrations tested. Aflatoxin G1 (AG1) and Aflatoxin G2 (AG2) displayed CYP-mediated toxicity similar to that of AB1. Aflatoxin M1 (AM1) was toxic only to the h1A2v2 and MCL-5 cell lines when tested up to 0.5 μM, while Aflatoxin M2 did not exhibit toxicity in any of the cell lines when tested up to 0.25 μM. Colchicine, which is directly toxic, was used as a positive control in these assays. In conclusion, based on results with these cell lines, AB1, AG1, and AG2 toxicities are CYP-generated, and are mediated principally by CYP1A2 and to a lesser extent by CYP3A4.

91 COAL DUST IS A MODIFIER OF PULMONARY CYP1A1 INDUCTION IN RATS.

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Cytochrome P4501A1 (CYP1A1) plays an important role in pulmonary carcinogenesis. Pulmonary CYP1A1 metabolizes inhaled exogenous agents, such as cigarette smoke-associated polycyclic aromatic hydrocarbons, into potent carcinogens. We hypothesized that intrapulmonary deposition of particulate might modify susceptibility to inhaled carcinogens by modifying induction and activity of pulmonary CYP1A1. To test this hypothesis, male Sprague-Dawley rats were intratracheally instilled with 0, 2.5, or 10 mg of respirable coal dust or iron oxide. Saline was instilled as a control. Eleven days later, they were treated with the CYP1A1 inducer β-naphthoflavone (NF; 50 mg/kg IP) or vehicle (corn oil). Three days after that, they were sacrificed and the level of functional CYP1A1 in the lungs was measured as ethoxyresorufin-*O*-deethylase (EROD) activity. Lung CYP1A1 protein induction and localization of CYP1A1 protein to alveolar type II cells was assessed by dual immunofluorescence staining for CYP1A1 and cytokeratin 8 (a cytoskeletal protein expressed in type II cells). We found that pulmonary EROD activity was significantly greater in coal dust/NF and iron oxide/NF rats than in saline/NF rats. Increasing coal dust lung burden accentuated the enhancement of EROD induction while increasing iron oxide lung burden diminished the enhancement seen at the lower lung burden. Immunofluorescence demonstrated that within the alveolus, a smaller proportion of CYP1A1 staining was associated with alveolar type II cells in coal dust/NF or iron oxide/NF rats than in saline/NF rats. These findings demonstrate that pulmonary exposure to coal dust modifies the inducibility and localization of CYP1A1 in the lung. These results suggest that respirable coal dust is not a simple co-variable in mixed exposures but a complex modifier of CYP1A1 induction that alters CYP1A1 activity and localization in the lung.

92 INDUCTION OF CYTOCHROMES P450 1A1 AND 1B1 BY MOTORCYCLE EXHAUST PARTICULATE IN HUMAN BREAST CANCER MCF-7 CELLS.

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The effects of motorcycle exhaust particulate (MEP) on cytochrome P450 (P450)-dependent monooxygenases were determined using MCF-7 human breast cancer cells treated with organic extracts of MEP. Treatment with MEP extract caused concentration- and time-dependent increases of monooxygenase activity in S9 fractions. Treatment with 50 μg/ml MEP extract for 24 h increased BENZO(A)PYRENE hydroxylase and 7-ethoxycoumarin, 7-ethoxyresorufin and methoxyresorufin O-dealkylases activities in S9. Treatment with 1 or 10 μg/ml MEP extract for 24 h markedly enhanced catabolism of 17 β-estradiol in MCF-7

cells. Co-treatment of the cells with 2 μM α-naphthoflavone, a P450 inhibitor and arylhydrocarbon receptor antagonist, blocked the increase of BENZO(A)PYRENE hydroxylase activity induced by treatment with MEP extract alone. Immunoblot analyses of S9 proteins using a mouse monoclonal antibody 1-12-3 against rat P450 1A1 and a rabbit polyclonal antibody against human P450 1B1 revealed that MEP extract induced proteins immunorelated to P450s 1A1 and 1B1. RNA blot analysis of total RNA using a human P450 1A1 3'-end and a human P450 1B1 RT-PCR product cDNA probes showed that MEP extract increased the levels of P450s 1A1 and 1B1 mRNA hybridizable to the respective cDNA probes. Treatment with 10 μM BENZO(A)PYRENE, a component of MEP extract, for 24 h induced catalytic activity, protein, and mRNA of P450s 1A1 and 1B1 in MCF-7 cells. Treatment with MEP extract increased P450s 1A1 and 1B1 proteins and mRNA levels in NCI-H322 human lung carcinoma and CL5 human lung adenocarcinoma cells. The extract also increased P450 1A1, but not P450 1B1, protein and mRNA in HepG2 human hepatoma cells. The present findings demonstrate that MEP extract has the ability to induce P450s 1A1 and 1B1 in the estrogen-responsive MCF-7 cells. Induction of the carcinogen- and estrogen-metabolizing P450s 1A1 and 1B1 may be an important factor to consider in assessing the potential health effects associated with human exposure to ME.

93 REGIONAL INDUCTION OF CYP1A1 PROTEIN DURING TREATMENT OF RATS WITH MIXTURES OF PCB126 AND PCB153.

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Liver enzyme induction by various inducers is regional and has all-or-none, switch-like characteristics *in vivo* and *in vitro*. We have evaluated enzyme induction and liver tumor promotion of a mixture of planar (PCB126) and non-co-planar (PCB153) PCBs in Fisher rats using a modified Ito initiation-promotion study. Starting two weeks after DEN initiation, rats were gavaged 3 times per week with PCBs. In a second study, enzyme activity for CYP1A1 was estimated in liver microsomes from Sprague-Dawley rats treated with PCBs for 13 to 30 weeks. In the Ito study, concentrations of PCBs in liver were determined for rats treated with mixtures or with either compound alone. At moderate doses, there was a more than additive increase in CYP1A1 activity in the multi-week dosing study and an increased liver concentration of PCB153 in mixture treated rats in the Ito study. At moderate to high doses, there were less than additive increases in number and area of GST-P+ foci. In the Ito study, exposure to PCB126 caused regional induction of CYP1A1, starting in the centrilobular region at 0.1 μg/kg/day and progressing outward until all the liver was induced at 10.0 μg/kg/day. In the high dose PCB126 rats that also received a high dose of PCB 153 (10, 000 μg/kg/day), the PCB153 concentrations reached 76 μg/g liver, 3-fold higher than those treated with PCB153 alone. In the mixture treated rats, the pattern of induction changed. Instead of complete induction of CYP1A1 throughout the liver as in the high dose PCB 126 rats, the pattern was regional showing preferential periportal staining, a reversal of the normal centrilobular pattern. This unexpected regional interaction may correlate with the inhibitory interactions for GST-P+ foci. This will also be studied in an *in vitro* switching model (CT French et al., this meeting). This work was supported by contract #1467 from the American Chemistry Council and NIEHS grants SBRP ES05949, KO8 ES00314 and KO8 ES00380.

94 INFLUENCE OF SIGNAL TRANSDUCTION PATHWAYS ON TCDD-MEDIATED *CYP1A1* GENE EXPRESSION.

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Cytochrome P450 1A1 (CYP1A1) catalyzes the hydroxylation of a variety of xenobiotic compounds and is strongly induced by halogenated and polycyclic aromatic hydrocarbons. Protein kinase C (PKC) has been implicated in the regulation of this response, since 1) several PKC inhibitors have been shown to inhibit induction by Ah receptor ligands, and 2) phorbol esters are synergistic to TCDD-mediated induction of *CYP1A1*. To define the role of PKC in regulation of the *CYP1A1* gene, we examined the effect of specific PKC inhibitors on this response. Experiments were conducted using the human HepG2 TV101L cell line, which stably expresses the firefly *luciferase* gene under control of the *CYP1A1* promoter (-1612 bp). TV101L cells were treated with 5 nM TCDD for 6 hours in the presence or absence of staurosporine or each of several specific PKC inhibitors (GF109203X, Gö6983, and Gö6976). TCDD alone did not significantly stimulate PKC activity. Phorbol 12-myristate 13-acetate (PMA) caused a 2- to 5-fold increase in luciferase activity both in the absence of TCDD (untreated compared to PMA treated TV101L cells) and the presence of TCDD (TCDD treated compared to TCDD and PMA cotreated TV101L cells). TV101L cells cotreated with TCDD and staurosporine exhibited a dose-dependent inhibition of PKC activity and of *CYP1A1*-luciferase activity at similar staurosporine concentrations. However, TV101L cells cotreated with TCDD and a specific PKC inhibitor showed a difference of approximately one order of magnitude between inhibitor concentrations necessary for dose-dependent inhibition of PKC activity and inhibitor concentrations necessary for dose-dependent inhibition of *CYP1A1*-luciferase activity. The magnitude of this difference was the same for TV101L cells cotreated with TCDD, PMA, and each of the specific PKC inhibitors. Combined, these results suggest that the actions of PKC may not constitute the sole component of *CYP1A1* regulation. (Supported in part by USPHS grant ES10337.)

95 HIGHER STABILITY OF TCDD-REGULATED CYP1A1 AND CYP1B1 mRNA IN THE MURINE AH RECEPTOR-DEFICIENT HEPA VARIANT THAN IN THE WILD TYPE HEPA-1C1C7 CELL LINE.

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The induction of CYP1A1 and CYP1B1 mRNA by 2, 3, 7, 8 tetrachloro-dibenzo-*p*-dioxin (TCDD) in Hepa-1 cells, wild type (WT) and the low Ah receptor (AhR) level variant (LA1) was examined. The kinetic analysis of the induction showed major differences related to the stability of the AhR and its two regulated genes. TCDD treatment of Hepa cells results in increased transcription of CYP1A1 and CYP1B1 with a subsequent down regulation of the AhR. We show here that nuclear translocation of the receptor is a pre-quisite for this depletion and that simultaneous treatment with actinomycin D reversed the TCDD-activated loss of AhR in WT. This indicates that continuous transcription is necessary to maintain receptor degradation by an apparently labile protease. In LA1 cells, however, AhR proteolysis was high in absence of TCDD treatment, and actinomycin D treatment stablized the cytosolic AhR levels only in LA1 cells, suggesting that the transcription-sensitive AhR degradation in LA1 cells is cytosolic rather than nuclear and might have contributed to the 10-fold lower constitutive AhR levels in LA1 variant. The transcription of CYP1A1 and CYP1B1 mRNA by TCDD in WT cells each exhibited bursts of activity in the initial hour which were about 3-fold greater than in LA1 cells, approximately in proportion to the respective nuclear AhR levels after 1 hr. However, the induced mRNA levels in LA1 exhibited a slow and prolonged increase reaching 50-80 percent of the WT steady state levels by 20 h. The decay of both CYP1A1 and CYP1B1 mRNA following inhibition of transcription by actinomycin D was much slower in LA1 cells. This slower mRNA turnover therefore, compensates for lower initial transcription rate and allows LA1 to gradually approach WT steady state levels. Thus, altered regulation of the AhR responsive genes in LA1 may result from the instability of the AhR due to this causative protease redistribution.

96 EFFECTS OF POLYCYCLIC AROMATIC HYDROCARBONS AND POLYCHLORINATED BIPHENYLS ON INDUCTION OF CYP1A1, CYP1A2, AND CYP1B1 IN LIVER AND LUNG TISSUES IN ARYLHYDROCARBON RECEPTOR KNOCK-OUT MICE.

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CYP1A1 and CYP1B1 are the most important enzymes in the initial step of oxidation of polycyclic aromatic hydrocarbons (PAHs) to form reactive intermediates and ultimate carcinogens. Arylhydrocarbon receptor (AhR) knock-out mice are shown to be resisitant to BENZO(A)PYRENE (B[a]P)-induced carcinogenesis, probably due to the inabilities to activate B[a]P by specific P450s in these mice. It is interesting to know which P450s are induced by PAHs and polychlorinated biphenyls (PCBs) in AhR(+/+) and (-/-) mice. These mice were injected i.p. with several PAHs and PCBs and were sacrificed at 3 days after the treatment. Levels of mRNA of Cyp1a1, Cyp1a2, and Cyp1b1 in liver and lung tissues and activities of liver microsomal P450s were measured. Cyp1a1 mRNA was very low in liver and lung tissues in untreated mice and was highly induced in AhR(+/+) mice by PAHs. 3, 4, 3', 4'-Tetrachlorobiphenyl induced liver and lung Cyp1a1 in AhR(+/+) mice, whereas PCB mixtures induced Cyp1a1 mRNA only in the liver. Cyp1a2 mRNA was increased with 3-methylcholanthrene and Kanechlor 500 in AhR(+/+) mice and was not detected in lung. Cyp1b1 was expressed constitutively in liver and lung tissues and was induced by PAHs and TCB in AhR(+/+) mice. Liver microsomal activities to oxidize several xenobiotics and carcinogens were found to be good indicators for the induction of individual P450s by PAHs and PCBs. Our results support that Cyp1a1, Cyp1a2, and Cyp1b1 are differentially induced in liver and lung tissues of AhR(+/+) mice by PAHs and PCBs through AhR and these may be related to the occurrence of tumor formation caused by PAHs in the target organs. (Supported in part by Grants from the Ministry of Education, Science, and Culture of Japan, the Ministry of Health and Welfare of Japan, and the Petroleum Energy Center.)

97 CYTOCHROME P450 2E1(CYP2E1) IS THE PRIMARY ENZYME RESPONSIBLE FOR ETHYL CARBAMATE (EC) METABOLISM: STUDIES USING CYP2E1-NULL MICE.

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EC is a fermentation product that is found in alcoholic beverages and some foods. It has been suggested that CYP2E1 is involved in EC metabolism. To directly assess the role of CYP2E1, EC metabolism was compared in CYP2E1-null (KO) and wild type (WT) mice. Carbonyl-14C-EC was administered (po) to male KO and WT mice at 10 or 100mg/kg. Mice were placed in metabolism cages that allowed for the quantitation of expired CO2, organic volatiles, urine, and feces. 14C derived from EC in tissues were dose-dependent and significantly higher in the KO *vs.* WT mice. Present data showed that CPY2E1 is the primary enzyme responsible for the metabolism of EC to CO2, however, other enzymes were involved. To identify these enzymes, the metabolism of 10mg/kg EC was compared in KO and WT mice pretreated with the CYP inhibitor 1-aminobenzotriazole (ABT, 50mg/kg ip) or the esterase inhibitor paraoxon (PAX, 1mg/kg ip). ABT resulted in significant inhibition of EC metabolism to CO2 in both genotypes. Moreover, higher levels of 14C derived from EC were detected in the tissues of ABT or PAX pretreated mice. In conclusion, these data demonstrated that CYP2E1 is the primary enzyme responsible for the metabolism of the carbonyl moiety of EC to CO2, however, other CYPs and esterases play a less significant role. The table below summarizes EC disposition at 24 hr after dosing:

	100mg/kg EC		10mg/kg EC		ABT+10mg/kg EC		PAX+10mg/kg EC	
	WT	KO	WT	KO	WT	KO	WT	KO
CO_2	95.0±5.2	46.1±5.0[a]	91.9±2.7	37.6±3.0[a]	13.0±2.3[a]	14.6±0.9[a]	96.0±1.2	35.1±1.7[a]
Organic Volatiles	0.5±0.2	2.6±0.9[a]	0.2±0.1	3.8±1.0[a]	3.6±0.1[a]	3.2±1.6[a]	0.7±0.1[a]	4.9±0.1[a]
Urine	3.5±1.2	3.9±0.8	2.3±0.4	5.6±2.1[a]	4.8±0.6[a]	4.2±0.3[a]	0.2±1.0[a]	1.1±1.2[a]

Values are the mean±SD of 3-4 mice. [a] and [b] denote statistically different values (P≤0.05) from corresponding WT and EC-only, respectively.

98 CYP1A1(-/-) KNOCKOUT MICE, WHICH HAVE A FUNCTIONAL AROMATIC HYDROCARBON RECEPTOR, ARE PROTECTED AGAINST DIOXIN, AS WELL AS BENZO(A)PYRENE, TOXICITY IN SPECIFIC TISSUES.

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Dioxin (2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin; TCDD) is a persistent environmental contaminant of unusual potency, owing to the fact that it is negligibly metabolized and it binds with high affinity to the aryl hydrocarbon receptor (AHR). The AHR is a ligand-activated transcription factor responsible for the up-regulation of numerous genes. It is thought that dioxin toxicity might result, at least in part, from chronic activation of one or more of these genes – leading to chronic oxidative stress; numbered among these genes are the cytochrome P450 1 (*Cyp1*) family members, *Cyp1a1*, *Cyp1a2* and *Cyp1b1*. Indeed, we have shown that the *Cyp1a2(-/-)* mouse is protected against dioxin-mediated hepatotoxicity. We also found that *Cyp1a1(-/-)* mice are more resistant to BENZO(A)PYRENE (BaP)-induced lethality, compared with *Cyp1a1(+/-)* mice. Although CYP1A1-dependent BaP-induced liver toxicity and metabolism do not correlate with CYP1A1-independent formation of BaP-DNA adducts. We then treated congenic *Cyp1a1(+/+)* or *Cyp1a1(-/-)* mice with a single high dose of dioxin (200 μg/kg, i.p.) and measured several endpoints of dioxin toxicity. First, all male *Cyp1a1(+/+)* animals died within 4 weeks following treatment, whereas the male *Cyp1a1(-/-)* mice and female animals of both genotypes did not die. The remaining analyses were performed on the surviving female animals. Serum ornithine carbamoyltransferase activities were not significantly different between *Cyp1a1(+/+)* and *Cyp1a1(-/-)* mice. On the other hand, the *Cyp1a1(+/+)* female mice had significantly smaller thymuses and displayed splenic atrophy, compared with that in the *Cyp1a1(-/-)* females. These results suggest that, like the *Cyp1a2(-/-)* mouse, *Cyp1a1(-/-)* mice may show limited protection against dioxin toxicity, but that the degree of protection will likely be tissue-specific. ***–Supported, in part, by NIH grants P30 ES06096, and R01 ES10133 and ES08147.***

99 THE ROLE OF PON1-192 AND P450 PATHWAYS IN PHOSPHOROTHIOATE METABOLISM BY HUMAN LIVER.

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In order to be toxic to the nervous system phosphorothioate pesticides (OPs) require activation to the oxon by the cytochromes P450. The oxon may then be detoxified by the hydrolytic A-esterase, PON1. The balance between the activation and detoxication pathways may influence susceptibility to OP toxicity because it governs the concentration of circulating oxon. The aims of this study were define the influence of PON1-192 genotype and P450 expression on the capacity of human liver microsomes (n=27) to metabolise parathion and chlorpyrifos. The study showed wide ranges in hepatic expression of CYP3A4 (n.d.-147 pmol/mg protein) and CYP2C8 (0.013-0.259 pmol/mg protein). CYP3A5 was expressed in five (23%) of the twenty-two livers (6-77 pmol/mg protein). The correlations between parathion activation to paraoxon and expressed CYP3A4/5 and CYP2C8 proteins were significant ($p<0.05$), suggesting the involvement of these isoforms in the reaction. However, correlations between chlorpyrifos metabolism and expression of CYP3A4/5 and CYP2C8 were not significant. There were wide ranges in activity for paraoxon and chlorpyrifos-oxon hydrolysis for individuals with the same PON1-192 genotype. Individuals with the RR genotype preferentially hydrolysed paraoxon, while individuals with the R and Q genotype hydrolysed phenylacetate and chlorpyrifos-oxon at similar rates. Correlation analysis of oxon hydrolysis by human microsomes showed that the three phenotypes were not well resolved and therefore could not be used to predict PON1-192 genotype. Individual expression of the participating P450 isoforms were more important than PON1 in determining hepatic capacity to metabolise parathion. PON1-192 genotype did not relate to metabolic capacity towards chlorpyrifos and other factors may be more important determinants of toxicity to this phosphorothioate.

100 CYP2A6 AND P53 IMMUNOREACTIVITY INDUCED BY SMOKING IN NON-SMALL CELL LUNG CANCER PATIENTS.

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CYP2A6 is a well known enzyme to metabolize nitrosamines in tobacco smoke. CYP2A6 mRNA is also observed in bronchial epithelial cells. On the other hand, p53 mutation is one of the most common genetic alternations found in human tumors including those of lung. p53 gene mutations lead to a nuclear accumulation of the p53 protein. The incidence of this mutation is related to the smoking index of the patients. The elevated concentration of carcinogens may act to produce these p53 alterations. In this study, we examined 19 patients with non-small cell lung cancer (NSCLC) for CYP2A6 and p53 staining and correlate the findings with lifetime cigarette consumption. We also revealed the relationships between CYP2A6 and p53 immunoreactivity in NSCLC. [Materials and Methods] We examined 19 Japanese patients with NSCLC who underwent surgical resection at UOEH. There were 11 men and 8 women ranging in age from 52 to 83 years (mean, 67 years). Ten patients had stage I & II disease, and 9 stage III & IV. Histological typing of the tumors was performed according to the WHO classification (adenocarcinoma; 12 cases, squamous cell carcinoma; 7 cases). The amount of lifetime cigarette consumption was classified by Brinkman Index (BI; cigarettes/day x years). Immunohistochemical staining (IHC) was performed on 3 mm paraffin-sections. The monoclonal antibody MAB-2A6 (Gentest) and DO-1 (Oncogene Science Inc.,) were used for IHC. We defined as CYP1A1 positive cases when more than 10% of bronchial cells were stained and as positive cases when more than 10% of tumor nuclei were stained. [Results and conclusions] The CYP2A6 positive rate in NSCLC patients with BI of more than 1000 (86%; 6/7) was significantly higher than that with BI less than 1000 (33%; 4/12). The positive rate of p53 expression in NSCLC patients with BI of more than 1000 (86%; 6/7) was significantly higher than that with BI less than 1000 (25%; 3/12). There was no significant relation between CYP2A6 and p53 expressions. Smoking may induce CYP2A6 and p53 expressions.

101 IDENTIFICATION OF NOVEL POLYMORPHISMS IN THE 5'-UNTRANSLATED REGION OF HUMAN CYTOCHROME P450 1B1.

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The human cytochrome P450 1B1 (CYP1B1) is a dioxin-responsive CYP capable of metabolizing and activating a variety of procarcinogens into their ultimate carcinogenic forms. Expression of the CYP1B1 gene varies considerably among humans, which may influence individual susceptibility to cancer. In order to determine whether genetic differences in CYP1B1 contribute to variability in gene expression, DNA was isolated from human tissue specimens and the -1139 to +19 region (relative to the transcription initiation site) of CYP1B1 was surveyed for genetic polymorphisms by the methods of Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP) and DNA sequencing. Three novel genetic polymorphisms, designated G-787A, T-350C, and C-238T were identified and their genetic frequencies were determined by Restriction Fragment Length

Polymorphism (RFLP) analysis. The -1139 to +19 region of CYP1B1 was cloned into a reporter plasmid and variant alleles for each polymorphism were introduced by site-directed mutagenesis. Wild type and variant reporter constructs were analyzed for their ability to induce transcription *in vitro* in transiently transfected Human MCF-7 breast adenocarcinoma cells. None of the variant alleles demonstrated significant differences in constitutive or dioxin-induced gene expression compared to the wild type constructs. These results may indicate that variations in the expression of human CYP1B1 are not influenced by genetic factors in the immediate vicinity of the transcription initiation site. (Supported in part by USEPA R825808 and Multidisciplinary Research Pilot Program, University at Buffalo.)

102 INHIBITION OF TCDD-INDUCIBLE CYP1A1 EXPRESSION IN MURINE HEPA-1C1C7 CELLS BY O, P'-DDT.

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Cultured mouse hepatoma Hepa-1c1c7 cells were treated with either o, p'-DDT or TCDD or in combination to assess the role of o, p'-DDT on CYP1A1 expression. o, p'-DDT alone did not affect CYP1A1-specific 7-ethoxyresorufin O-deethylase (EROD) activity. In contrast, the TCDD-inducible EROD activities were markedly reduced upon concomitant treatment with TCDD and o, p'-DDT in a dose dependent manner. Treatment with ICI 182.780, an estrogen-receptor antagonist, did not affect the suppressive effects of o, p'-DDT on TCDD-inducible EROD activity. The TCDD-inducible CYP1A1 mRNA levels were markedly suppressed upon concomitant treatment with TCDD and o, p'-DDT that is consistent with their effects on EROD activity. A transient transfection assay using dioxin-response element (DRE)-linked luciferase and electrophoretic mobility shift assay revealed that o, p'-DDT reduced transformation of the aryl hydrocarbons (Ah) receptor to a form capable of specifically binding to the DRE sequence in the promoter of the CYP1A1 gene. These results suggest that the down regulation of CYP1A1 gene expression by o, p'-DDT in Hepa-1c1c7 cells might be an antagonism of the DRE binding potential of the nuclear Ah receptor but is not mediated through the estradiol receptor [This work was supported by KFDA Grant (ED2001-14) and RCPM].

103 INACTIVATION OF CYTOCHROME P450 2E1 BY CAPSAICIN.

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Capsaicin is the principle pungent component of "hot peppers" and of pepper spray products used for self defense. Previous research has demonstrated that dihydrocapsaicin interacted with hepatic drug-metabolizing enzymes, specifically P450 2E1, and caused a loss of activity and protein adduct formation. In this study, the mechanism of P450 2E1 inactivation by capsaicin has been characterized. A 2E1 enzyme assay composed of recombinant human P450 2E1, NADPH, and p-nitrophenol at 37°C was used. Enzyme activity was determined by spectrophotometric detection of 4-nitrocatechol, the hydroxylated product of p-nitrophenol. We observed both a time- and concentration-dependent decrease in P450 2E1 activity when capsaicin and NADPH were included in incubation mixtures. Increasing concentrations of capsaicin (up to 1 mM) decreased the rate of p-nitrophenol hydroxylation up to 90%. Approximately 43% of P450 2E1 activity was lost upon preincubation for 30 minutes in the presence of 100 μM capsaicin and NADPH. The rate of inactivation of P450 2E1 was 0.025 min^{-1}. The apparent K_i value was 23.8 μM. We have previously shown that glutathione (2mM) increased the metabolism of capsaicin by human liver microsomes. One hypothesis for this observation was that the metabolism of capsaicin by P450 2E1 produced a reactive intermediate, possibly through the oxygenation or dehydrogenation of the phenolic ring of capsaicin. Protection of CYP2E1 was also observed with the addition of reduced glutathione (1mM). These data suggested that capsaicin was an uncompetitive, mechanism-based inhibitor of P450 2E1, and that the electrophilic reactive intermediate that inactivated 2E1 was released from the active site of the enzyme. These studies indicate that exposure to capsaicin may elicit drug interactions by irreversible inhibition of a vital enzyme, CYP2E1. This work supported by a grant from the National Institute of Standards and Technology (Department. Commerce # 60NAN-BOD0006).

104 INHIBITION OF CYTOCHROME P450 METABOLISM BY METALLOPORPHYRINS.

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Manganese containing metalloporphyrins have been shown to possess a wide variety of antioxidant properties, including the scavenging of superoxide, hydrogen peroxide, lipid peroxides and peroxynitrite. These compounds are currently being developed as therapeutic agents in the treatment of stroke, organ transplant, and cancer. The goal of these studies was to determine whether these compounds interfer with cytochrome P450 metabolism, both *in vivo* and *in vitro*. Groups of 4 rats were treated with AEOL-10113 (0.5 mg/kg, SQ) and 24 hours later benzyloxyresorufin O-dealkylase (BROD)activity was assess from their liver microsomes. AEOL-10113 markedly depressed BROD activity by greater than 50%. Untreated rat liver microsomes were used to determine the *in vitro* concentration of AEOL-10113 that inhibits BROD activity by 50% (IC_{50}). AEOL-10113 exhibited an IC_{50} of 0.2 μM in the BROD assay. The maganese containing metalloporphyrin, AEOL-10113, is a potent inhibitor of cytochrome P450s. Given AEOL-10113's potent ability to scavenge reactive oxygen species, it is possible that these compounds scavenge the cytochrome P450 activated oxygen intermediate that is necessary for cytochrome P450 catalytic activity.

105 INHIBITORY EFFECTS OF GOLDENSEAL METHYLENEDIOXYPHENYL COMPOUNDS ON HUMAN CYTOCHROME P450S: CYP2C9, CYP2D6, AND CYP3A4.

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A large segment of the population is consuming phytomedicinals concomitant with conventional prescription or OTC drugs. The concurrent use of herbal medicinals carries a potential risk for adverse drug-botanical pharmacokinetic interactions, particularly as a result of inhibition of cytochrome P450s. Goldenseal (Hydrastis canadensis), a popular immunostimulant botanical, contains three methylenedioxyphenyl (MDP) alkaloids, berberine, (+ & -)-hydrastine, and hydrastinine. In human liver microsomes, both berberine and hydrastinine, inhibited (CYP2D6) bufuralol-1'-hydroxylation, (IC50 s of 15 and 30 uM respectively). Berberine also inhibited (CYP3A4) testosterone-6b-hydroxylation (IC50 of 120 uM. Of the two isomers of hydrastine, neither inhibited CYP2D6, both equally inhibited (CYP2C9) diclofenac-4'-hydroxylation (IC50 of 150 uM, and only the (-) isomer inhibited CYP3A4 (IC50 of 20 uM. Therefore at least 3 CYPs could be affected by MDPs contained in goldenseal. Examination of isozyme selectivity of inhibition by model MDP compounds piperonyl butoxide, safrole, isosafrole, and methylenedioxybenzene itself, indicate that it is highly compound dependent. Piperonyl butoxide inhibited all three (IC50 s of 25, 200 and 0.2 uM for CYP2C9, CYP2D6 and CYP3A4 respectively) whereas safrole and methylenedioxybenzene only inhibited CYP3A4 (IC50 s of 0.4 and 12 uM respectively). Isosafrole was a strong inhibitor of both CYP2D6 and CYP3A4 (IC50 s of 0.2 and 3 uM respectively). [For comparative purposes, quinidine showed an IC50 value of 0.05 uM towards CYP2D6, sulfaphenazole and phenytoin showed IC50 values of 3 and 300 uM respectively towards CYP2C9, and ketoconazole, phenytoin and carbamazepine showed IC50 values of 0.07, 80 and 300 uM respectively towards CYP3A4.]

106 CHARACTERIZATION OF NONSPECIFIC EFFECTS OF PHOSPHOROTHIOATE OLIGODEOXYNUCLEOTIDES ON CYTOCHROME P450 3A2 AND CYTOCHROME P450 2C11 IN MALE SPRAGUE-DAWLEY RATS.

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Phosporothioate oligodeoxynucleotides (PS ODNs) are being studied for treatment of various diseases and are designed to bind specific regions of a target gene to block translation of proteins. PS ODNs can also have nonspecific effects in rodents that lead to immune stimulation as demonstrated by the production of inflammatory cytokines. The purpose of our research is to study the nonspecific effects of PS ODNs on hepatic metabolism in rodents. Certain inflammatory cytokines (IL-2, IL-6, IL-12 and IFN) are known to reduce the activity of specific cytochrome P450 isozymes. Cytochrome P450 3A2 and 2C11 are predominate isoforms of the liver and if altered pose concern for potential drug-drug interactions. Six to eight week old male Sprague-Dawley rats were injected subcutaneously with 5, 15, or 50 mg/kg of ISIS 2105 every other day for a total of three doses. Twenty-four to 30 hours after the last injection, livers, spleens and blood samples were collected. Microsomes were isolated from whole livers and hepatic cytochrome P450 (CYP 3A2 and CYP 2C11) activity was determined by a testosterone hydroxylation assay. Spleen weights were used as a marker for immune stimulation. Plasma attained after a 50 mg/kg dose of PS ODN were measured for cytokine (IL-6) levels by ELISA. Dose-dependent suppression of CYP 3A2 and CYP 2C11 activity was observed, with approximately a 3-fold decrease at the highest dose (50 mg/kg). The decrease in CYP P450 activity was correlated with a dose-dependent increase in spleen weight. Furthermore, IL-6 levels were significantly elevated (approximately 2.5 fold over control) 8 hours post injection. Decreased activity of CYP 3A2 and

CYP 2C11 suggests that cytokines released in response to PS ODN treatment can suppress hepatic metabolism. Additional studies are pending to further study the direct and indirect effects of PS ODN on hepatic metabolism.

107 CYTOCHROME P450 3A2 ANTISENSE MORPHOLINO OLIGOMER AVI-4472: A 14-DAY TOXICITY STUDY IN MALE RATS.

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Cytochrome P450 (CYP) 3A2 is recognized as the rat ortholog of the human CYP3A4 enzyme, which plays a role in the metabolism of more than half of clinically relevant xenobiotics. Phosphorodiamidate Morpholino Oligomers (PMO) are third generation antisense agents that have immense therapeutic potential. The objectives of this study were to: (1) evaluate the toxicity and tissue distribution of CYP3A2 antisense PMO AVI-4472 following daily intraperitoneal injections to male rats for 14 days at two dose levels; and (2) to study the reversibility of observations following a 14-day recovery period. Male Sprague-Dawley rats were injected with vehicle, 3.0 or 30.0 mg/kg/day AVI-4472 intraperitoneally for 14 days. Results indicate that animals in all groups maintained good general health and there were no significant differences in body or organ weights between any groups. Hematological examination revealed an increase in monocyte count to 522±212 in the high dose group compared with 192±90 in the vehicle group ($p<0.05$). This index returned to normal following 14 days of recovery. There was a decrease in total bilirubin content to 0.35±0.08 mg/dl in the high dose group compared with 0.60±0.23 mg/dl for the vehicle group ($p<0.05$). This index did not recover following 14 days of no treatment. Organ distribution of the PMO was identified in all organs where the target enzyme is detectable, namely kidneys, liver and small intestines (descending order of recovery) at the end of the 14-day treatment period. PMO was also detectable in these organs at the end of the additional 14-day no-treatment period. We conclude that it is feasible to administer AVI-4472 systemically to male rats for long-term suppression of the CYP3A2 enzyme.

108 ALTERATIONS IN HISTONE ACETYLATION ARE ASSOCIATED WITH PHENOBARBITAL INDUCTION OF THE RAT CYP2B2 GENE.

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Phenobarbital (PB) exposures result in the marked induction of a number of biotransformation enzymes in the liver of mammalian organisms, including certain cytochrome P450s (CYP). This process is mediated by the activation of the CAR/RXR nuclear receptor complex and is inhibited by the phosphatase inhibitor okadaic acid. Remodeling of chromatin structure is an important event associated with the activation of specific genes by chemical inducers. Previously, it was reported that PB-induced transcriptional activation of the rat CYP2B2 gene involved tissue-specific alteration of chromatin structure surrounding a key regulatory element, termed the PB responsive unit, or PBRU. We used a chromatin immunoprecipitation assay and a primary rat hepatocyte culture system to demonstrate that PB exposures result in enhanced histone H3 acetylation, specifically within the PBRU region of the CYP2B2 gene. The changes noted in H3 acetylation were correlated with the binding of RXR_{α} to the PBRU. Furthermore, we demonstrate that okadaic acid inhibits the changes in histone acetylation and blocks the recruitment of RXR_{α} to the PBRU. Characterizing the molecular events associated with xenobiotic induction will enhance our ability to predict the biological and toxicological impact of chemical exposures in affected cells. (Supported by NIH grant GM32281 and the Burroughs Wellcome Fund)

109 EFFECTS OF MATERNAL DIETARY VITAMIN E SUPPLEMENTATION ON DEVELOPMENTAL BIOLOGY AND PHENYTOIN EMBRYOPATHIES.

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The antioxidant vitamin E (VE) given *i.p.* protects pregnant mice from embryonic DNA oxidation and teratogenicity caused by the reactive oxygen species (ROS)-initiating drug phenytoin (PHT). Also, maternal dietary supplementation with low (0.1-2.0%) but not high (10%) levels of VE reduces both endogenous and PHT-enhanced DNA oxidation. Here, we examined the effect of maternal dietary VE (dl-α-tocopherol-acetate) supplementation on normal embryonic development and PHT teratogenicity. Virgin CD-1 females were placed on either a normal or VE-supplemented (0.1%, 2.0%, or 10% [w/w]) diet for 4 weeks and mated. Dams were administered PHT (65 mg/kg *i.p.*) or its saline vehicle on gestational days (GD) 12 and 13 at 0900 hr. In vehicle controls on GD 19, spontaneous fetal resorption incidence was reduced 84% by 0.1% dietary VE ($p<0.006$), less so (61%) by 2% VE ($p<0.05$), and least by 10% VE ($p>0.05$). In PHT-treated animals, 0.1% VE reduced PHT-enhanced fetal resorptions by 68% ($p<0.02$), but this protective effect was lost at higher VE doses. PHT-enhanced fetal weight loss ($p<0.01$) was reduced to the level of vehicle controls at 2.0% and 10% VE. VE did not significantly alter PHT-initiated fetal cleft palate incidence, which was greater than in vehicle controls ($p<0.02$), and higher VE diets of 2.0% and 10% non-significantly enhanced postpartum lethality in a dose-dependent fashion. The VE effects on resorptions are congruent with those on DNA oxidation, where 0.1% VE, but not higher doses, provided optimal protection from both endogenous and PHT-enhanced DNA oxidation. The protection by VE suggests ROS involvement in at least some PHT embryopathies, possibly involving embryonic DNA oxidation, but the biphasic nature of VE protection suggests caution in the design of therapeutic strategies. The results from vehicle controls show that even endogenous levels of ROS are embryopathic, suggesting developmental benefits from maternal dietary VE supplementation (Support: CIHR)

110 DEVELOPMENTAL TOXICITY OF DI- AND TETRACHLOROETHANE AND DICHLOROPROPANE IN THE RAT WHOLE EMBRYO CULTURE SYSTEM.

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Disinfection of drinking water with chlorine results in numerous chlorinated byproducts (DBPs). Among these are the di-substituted propanes and the di-and tetra- substituted ethanes. We chose to evaluate the developmetal toxicity of 1, 3-dichloropropane (1, 3DP), 2, 2-dichloropropane (2, 2DP), 1, 1-dichloroethane (1, 1DE) and 1, 1, 2, 2-tetrachloroethane (TCE) in rat whole embryo culture (WEC) due to their presence in drinking water and a lack of developmental toxicity data available for these compounds. Gestational day (GD) 9 rat embryos were exposed to 2.5-7.6 mM 1, 3 DP, 8.7-19.4 mM 2, 2 DP, 8.9-23.8 mM 1, 1DE, or 0.5-2.9 mM TCE for 48 hours and then evaluated. All compounds were dysmorphogenic in rat WEC. 1, 3 DP was dysmorphogenic at all concentrations >3.8 mM and embryolethal at concentrations >5.1 mM. Anomalies were opened neural tube, rotational and eye defects. DEVSC, HL, SN and CRL were significantly reduced at concentrations >3.8 mM. 2, 2DP was dysmorphogenic in rat WEC at concentrations >11.6 mM and embryolethal at concentrations >14.5 mM. Anomalies were predominantly open neural tube, heart defects and rotational defects. DEVSC was significantly reduced at concentrations >11.6 mM. 1, 1DE was significantly dysmorphogenic at 17.9 mM but also significantly embryolethal (35%). The anomalies were eye defects but there was overall delayed embryonic development at the higher exposure concentrations. TCE was dysmorphogenic at concentrations >1.4 mM and embryolethal at concentrations >2.4 mM. Anomalies were primarily heart, eye and rotational defects. The relative developmental toxicity for the four compounds was TCE>1, 3DP>2, 2DP>1, 1DE. The pattern of anomalies was inconsistent between the 4 compounds suggesting that their toxicity is probably expressed through different mechanisms. The concentrations of these DBPs required to induce dysmorphology are much higher than those for the haloacetic acids, another class of DBPs that we have studied extensively. This abstract does not reflect EPA Policy.

111 A NEW ANIMAL MODEL FOR AUTISM: EFFECTS OF EARLY EXPOSURE TO SODIUM VALPROATE IN MICE.

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Early exposure to environmental agents may have a role in the etiology of pervasivedevelopmental disorders (PDD) including autism. Such exposure may cause neurobehavioral retardation or regression (wherein an acquired behavior is lost), or intrusions (wherein acquired behaviors are overshadowed by other behaviors abberant in form or frequency). Teratogenic effects of the antiepileptic drug sodium valproate (VPA) have been well documented in the form of neurological deficits and decreases in Purkinje cell number, which have been associated with autism. In the present study, PND 14 male BALB/c mice were tested for skills corresponding to cerebellar development. Following initial testing, pups were treated with 200 mg/kg sc sodium valproate (VPA), retested on these tasks 24hr, 2, 3 and 4 days later and examined for emergence of habituation of motor activity and spatial learning ability on PND 23-26. Treatment with VPA on day 14 produced a regression in mid-air righting as well as an established negative geotropic response; that is, while pups had developed these abilities before treatment, these behaviors were lost for up to 72 hours after VPA administration. In addition, treated pups showed a delay in navigation of a water maze task with no alterations in the habituation of motor activity. VPA-induced changes in developmental behaviors were not observed when

VPA (200 mg/kg sc) was administered daily on days 13-16 of gestation. Finally, in order to investigate regressions on later emerging skills such as spatial learning, PND 23 pups were trained for 4 days on the water maze and also allowed to habituate to an open field, then treated with VPA (200 mg/kg) on day 26. A regression in water maze ability was seen 48 hours later, manifested as an increase in escape latency in the water maze compared to those days prior to treatment. These data propose a new behavioral model for PDD which can be used to assess the role of known teratogens on behavioral development. (Supported by ES 07148 and by Johnson and Johnson).

112 EFFECTS OF LOW LEVEL TECHNICAL CHLORDANE ON SELECTED SEX STEROID MEDIATED PARAMETERS IN PREPUBERTAL RATS.

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Sponsor: R. Dalvi.

Technical chlordane is a highly persistent toxic environmental pollutant and a formerly used pesticide. Humans and other mammals are being exposed to low levels of chlordane and their long-term effects during critical developmental periods are not well documented. This study evaluated the effects of technical chlordane on sex steroid mediated parameters (anogenital distance [AGD], testicular descent, preputial separation, testes, seminal vesicles, prostate, testosterone, uterus and ovaries) of prepubertal rats in a multigenerational study. Sprague-Dawley rats, F1 adult and their F2C offspring were exposed, pre- and postnatally to daily levels of chlordane (0.0, 0.125, 0.25 and 1.0 mg/kg) until termination. F2C rats on PND 1 manifested increases ($p<0.05$) in AGD, a biomarker of androgenicity. Treatment also delayed the ontogeny of balano-preputial separation and testicular descent (androgenic biomarker) in F2C males. While in females, chlordane delayed vaginal opening (an estrogen dependent parameter). Plasma testosterone concentrations on PND 30 were reduced ($p<0.05$) in treated males but not females. The physical maturational parameters (balano-preputial separation, testicular descent and vaginal opening) correlated with changes in plasma testosterone concentrations. Dose related reductions occurred in absolute and relative organ /brain weight ratio of testes, uterus and liver. In conclusion, chlordane exhibited androgenic-like activities *in utero*, and both anti-androgenic (males) and anti-estrogenic (females) responses during prepubertal development. Therefore, humans may be at risk from chlordane as an endocrine disruptor. (Supported by: MHPF/ATSDR #U50/ATU3922660008).

113 EMBRYOTOXICITY ASSOCIATED WITH SPERMATOZOA BENZO(A)PYRENE-DNA ADDUCTS TRANSFERRED TO FERTILIZED EGGS.

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Sperm benzo(a)pyrene-DNA adducts can be transferred to fertilized embryos. Using 33P-postlabeling, we have previously identified a DNA adduct [(+) anti-BPDE-deoxyguanosine] in trout sperm treated with benzo(a)pyrene diol epoxide (BPDE). When BPDE-treated sperm were used to fertilize untreated trout eggs, the same adduct was found at 24h. To assess developmental effects, trout sperm were incubated with 50 ug/ml BPDE or saline for 1h, washed and used to fertilize untreated trout eggs. For 10d, and at 15d post-fertilization, eggs were examined microscopically. Eggs hatched at 20d and fry were weighed. At 24h post-fertilization, eggs in both groups had visible cell masses indicating 100% fertilization. By 72h, gastrulation was observed in 96% of PBS embryos but was delayed in embryos from BPDE-treated sperm with gastrulation in only 77%. At 5d, approx. 95% of PBS embryos had formed a complete body axis compared to approx. 60% of BPDE embryos. Between days 6 and 10, approx 85% to 95% of PBS embryos (n=50 each day) had normal development with 98% having eye spots & beating hearts on day 10. In contrast, only approx. 50% of BPDE embryos (50 each day) appeared normal. Of the abnormals, half had no noticeable development while the others had truncated body axes with disorganized cell masses. At 15d, 95% (of 212) PBS embryos had normal development compared to 32% (of 143) from BPDE-treated sperm. Eggs from PBS-treated sperm hatched at 72% (1369 of 1912 eggs) with normal trout fry, while only 8% (85 of 1137 eggs) from BPDE-treated sperm hatched. Those that hatched appeared & weighed the same as PBS fry. Non-viable eggs from BPDE-treated sperm had varying forms of abnormal development ranging from none, to elongated masses of cells, to various degrees of truncated development. Sperm adducts did not affect fertilization rate, but were associated with embryotoxicity: delayed gastrulation; incomplete body axis formation; abnormal development; and decreased hatching. Assistance provided by the Bodine State Trout Hatchery, IN Department. of Natural Resources.

114 CONTRASTING DEVELOPMENTAL ROLES FOR ATAXIA TELANGIECTASIA-MUTATED (ATM) IN MODULATING THE EMBRYONIC RESPONSE TO ENDOGENOUS AND XENOBIOTIC-ENHANCED REACTIVE OXYGEN SPECIES (ROS) IN ATM KNOCKOUT MICE.

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The ATM protein is a key ROS sensor and signal transducer in the genotoxic stress response pathway, and can trigger p53-dependent DNA repair. We have shown that pregnant Atm-knockout mice are more sensitive to the embryopathic effects of ionizing radiation, and here we determined if they are similarly more susceptible to the embryopathic effects of the ROS-initiating drug phenytoin. Female wild-type (+/+) or heterozygous (+/-) Atm knockout mice were mated with +/- males, and dams were treated with phenytoin (65 mg/kg ip) or its saline vehicle on gestational days (GD)11 and 12 (plug=GD1). Resorptions and fetuses were genotyped and characterized on GD19. With untreated (vehicle controls) +/- dams, fetal body weight was lower in homozygous Atm null (-/-) fetuses compared to +/- and +/+ littermates ($p<0.05$). Independent of treatment, there were opposite Atm gene dose-dependencies in pre- and post-implantation death. Implantations were 45% lower in +/- than +/+ dams ($p<0.05$). In contrast, in +/- dams, no resorptions had a -/- genotype, compared to a substantial incidence with +/- (45%) or +/+ (55%) genotypes in +/+ and +/- dams ($p<0.05$). This strain was relatively resistant to phenytoin embryopathies. In +/+ dams, phenytoin did not cause fetal body weight loss nor enhance resorptions or postpartum lethality. However, with +/- dams, fetal body weight loss was enhanced by phenytoin in -/- Atm fetuses compared to both their +/- and +/+ phenytoin-exposed littermates ($p<0.05$), and to -/- vehicle controls ($p<0.05$). Conversely, phenytoin-initiated postpartum lethality was increased in offspring of +/+ compared to +/- dams ($p<0.05$). These results suggest that ATM plays important and contrasting developmental roles, and provide the first evidence that ATM affords protection against some, while enhancing other, embryopathic effects of ROS-initiating teratogens. (Support: Canadian Institutes of Health Research)

115 TRICHLOROETHYLENE, TRICHLOROACETIC ACID AND DICHLOROACETIC ACID: DOES *IN UTERO* EXPOSURE TO THESE CHEMICALS AFFECT EYE DEVELOPMENT?

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Maternal exposure to high doses of TCE has been implicated in eye malformations in fetal rats, primarily micro-/anophthalmia. Coincident with a cardiac teratology study of TCE and its metabolites, TCA and DCA, the potential for these compounds to induce ocular terata was examined. Pregnant, Sprague-Dawley rats were orally treated on gestation days 6-15 with bolus doses of either TCE (500 mg/kg), TCA (300 mg/kg), DCA (300 mg/kg) or the prototypical teratogen, all-*trans* retinoic acid (RA, 15 mg/kg). The heads of gestation day 21 fetuses were not only examined grossly for external malformations, but nearly 600 fetal heads were sectioned using a modified Wilson's technique and subjected to computerized morphometry that allowed for the quantification of lens area, globe area and interocular distance. Gross ocular malformations were essentially absent in all treatment groups except for the RA group in which 26% of fetuses exhibited micro-/anophthalmia. Lens area, globe area and interocular distance were significantly reduced in the TCA, DCA and RA treatment groups, but not the TCE treatment group. However, the reduced body weights of TCA-, DCA- and RA-treated fetuses appear sufficient to explain the changes in these dependent variables. Based upon these preliminary results, neither TCE nor its acid metabolites can be characterized as ocular teratogens. With respect to TCE, this differs from earlier reports generated with a different rat strain and larger doses.

116 EVALUATION OF ULTRASOUND TRANSIT-TIME FLOWMETRY TO MEASURE UTERINE BLOOD FLOW DURING PREGNANCY IN THE RAT.

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Uterine blood flow (UBF) is the major determinant of oxygen and other essential nutrient transport to the fetus. Alterations of UBF during pregnancy may impair fetal development. In recent years, many techniques have been developed for organ blood flow measurement, including ultrasound flowmetry. The aim of the present

study was to evaluate this technique for quantifying UBF in pregnant rats. Maternal UBF, and heart rate were measured on sperm-positive Sprague-Dawley rats on Gestation Day (GD) 21 by using a flowmeter and 1 mm flow probes (1RB, Transonic Systems, Ithaca, NY). Mean absolute and relative UBF were 2.96 ± 1.60 ml/min and 0.029 ± 0.015 ml/min/g of gravid uterus, respectively, and heart rate was 393 ± 36 rpm. These values were within the expected ranges reported in the literature. We then investigated the effects on UBF of phenylephrine, a postsynaptic alpha-adrenergic agonist agent which induces uterine vasoconstriction. Pregnant Sprague-Dawley rats on GD21 were given subcutaneously 12.5 mg/kg phenylephrine and UBF was measured continuously for up to 25 minutes after injection. We found that UBF decreased up to 91% when compared to the control values recorded before injection, whereas HR was unchanged. Overall our results indicated that the ultrasound transit time flowmeter system appears as a promising technique to determine UBF in pregnant rats and to identify negative effects induced by vasoconstrictive agents.

117 POSTNATAL INORGANIC LEAD EXPOSURE DECREASES THE NUMBER OF SPONTANEOUSLY ACTIVE MIDBRAIN DOPAMINE NEURONS AND ALTERS THE SENSITIVITY OF NUCLEUS ACCUMBENS NEURONS TO D1 RECEPTOR STIMULATION.

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The effect of postnatal lead (Pb) exposure on the electrophysiological activity of midbrain dopamine(DA) neurons was examined during postnatal development. Single-cell electrophysiological recordings were made in the substantia nigra (SN) and ventral tegmental area (VTA) of chloral hydrate anesthetized rats. 21-day-old male Sprague-Dawely rats were exposed to Pb- or Na-acetate in the drinking water for a period of 3 to 6 weeks or 7 to 13 weeks. This exposure resulted in a significant Pb-dependent decrease in the number of spontaneously active DA neurons at the time of recording. The 250 and 500 ppm level of Pb exposure produced a significant decrease in the number of spontaneously active DA neurons in both the SN and VTA. Apomorphine challenge during some electrophysiological recordings indicated that this decrease in activity was most likely not due to depolarization-inactivation. The effect of selected Pb exposure levels, 250 and 500 ppm, were examined during the postnatal period using tyrosine hydroxylase (TH) immuno-histochemistry to determine if Pb affected the survival of dopamine neurons within SN and VTA. TH immunohistochemical studies revealed that the reduction in the number of spontaneously active DA neurons in animals treated with 250 and 500 ppm Pb was not related to the physical loss of cells (e.g., necrosis or apoptosis). Using the same exposure protocol (250 ppm Pb for 3 to 6 weeks) microiontophoretic studies of neurons in the nucleus accumbens was conducted using micropipettes loaded with the D1 receptor agonist, SKF-38393. Nucleus accumbens neurons from Pb-treated animals were found to be significantly less sensitive to SKF-38393 relative to controls. These results indicate that exposure to Pb at clinically relevant levels has significant effects on the function of dopaminergic circuitry without causing dopamine neuron loss.

118 CRITICAL PERIOD FOR INCREASED NEONATAL MORTALITY INDUCED BY PERFLUOROOCTANE SULFONATE (PFOS) IN THE RAT.

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PFOS belongs to a class of fluorinated organic chemicals with broad industrial use. Despite a reduction in the manufacturing of products containing PFOS and many of its metabolic precursors, levels of accumulated PFOS in the environment warrant evaluation of its potential for toxicity. The present study investigated the critical period for increased neonatal mortality caused by PFOS. Timed-pregnant Sprague-Dawley rats were treated by oral gavage with 25 mg/kg PFOS (potassium salt in 0.5% Tween-20) for four consecutive days during either GD 2-5, 6-9, 10-13, 14-17 or 17-20. Controls were given vehicle daily throughout GD 2-20. Blood samples were taken the day after the last day of dosing to determine maternal PFOS levels. Dams were allowed to continue normal pregnancy through birth. Maternal weight gain was reduced in treated animals during dosing and returned to near control levels by term. Similar patterns were seen in food and water consumption. Average litter size was normal in all groups compared to controls with no significant difference in average pup weight on the day of birth. PFOS induced decreases in neonatal survival were seen for each group while controls remained at about 100% survival. Mortality increased as the dosing period fell later during gestation with the latest group (GD 17-20) approaching 100% mortality. All deaths occurred before PD 4 with most taking place during the first 24 hours. Growth retardation was observed in surviving pups of treated dams and gross dissection suggested underdeveloped lungs. A second study compared postnatal survival between dams treated with 100 mg/kg PFOS on GD 17 or 20. Both groups had a high rate of neonatal death with no significant difference between the two. Analysis of maternal PFOS levels are in underway. These results demonstrate that the critical period for PFOS induced neonatal mortality in the rat occurs near birth. This abstract does not reflect EPA policy.

119 MATERNAL AND DEVELOPMENTAL TOXICITY OF PERFLUOROOCTANE SULFONATE (PFOS) IN THE RAT.

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The maternal and developmental toxicity of PFOS, an environmentally persistent compound used as surfactants and insecticides, were evaluated. Timed-pregnant Sprague-Dawley rats were given 1, 2, 3, 5, or 10 mg/kg/day PFOS/K+ by gavage on GD 2 through term. Controls received 0.5% Tween-20 vehicle (1 ml/kg). Some rats were killed on GD 21 for teratological examination, others were allowed to deliver, and postnatal growth and development were monitored. PFOS levels in serum and liver were determined. Maternal weight gain was suppressed by PFOS, likely due to reduced food and water intake. Serum cholesterol, triglycerides, thyroxine (T4) and triiodothyronine (T3) in PFOS-treated dams were lower than those in controls, but TSH was not affected. PFOS did not alter the number of implantations or live fetuses at term, although small deficits in fetal weight were noted in the high dose groups. Cleft palate, anasarca, cardiac ventricular septal defect, and small/hemorrhagic lung were detected, primarily in the 10 mg/kg group. Live birth was observed in all groups; however, neonates in the 10 mg/kg group were moribund and died within 4-6 h. While newborns in the 5 mg/kg group appeared viable, >95% were found dead within 24 h; postnatal growth was retarded in the survivors. Cross-fostering of pups to control dams at birth did not improve the survival rate, nor adverse effect seen in control pups nursed by PFOS-exposed dams. Postnatal viability was greater in the lower dose groups and surviving neonates appeared to thrive, but mild hypothyroidism (only T4 reduction) and delays in eye opening were noted. These dose-dependent adverse effects will be compared to the body burdens of PFOS. Our results indicate both maternal and developmental toxicity of PFOS in the rat; while PFOS altered the thyroid status, this hormonal imbalance is not likely the sole contributor to neonatal mortality. This abstract does not reflect EPA policy.

120 PERFLUOROOCTANESULFONATE-INDUCED PERINATAL MORTALITY IN RAT PUPS IS ASSOCIATED WITH A STEEP DOSE-RESPONSE.

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To better define the dose-response of perinatal mortality observed in prior reproduction studies with perfluorooctanesulfonate (PFOS), female rats were administered potassium PFOS by oral intubation at dose levels of 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg/d for 42 days prior to mating to untreated males, during mating and through the end of gestation (cesarean section) or four days of lactation (natural delivery). At day 21 of gestation, fetuses from eight litters in the 0, 1.6, and 2.0 mg/kg/d dose groups were taken by cesarean section. All remaining dams were allowed to litter, and were sacrificed with litters on day 5 of lactation. For all or some of the groups, PFOS sera levels, serum lipids, glucose and thyroid hormones were measured at both time points. Milk cholesterol and liver PFOS were also measured on lactation day 5. Significant decreases in cholesterol and increases in LDL were observed in all fetuses from the 1.6 and 2.0 mg/kg/d PFOS dose groups on day 21 of gestation. On day 5 of lactation, dams had significantly decreased serum cholesterol at all dose levels, significantly decreased serum triglycerides in the 1.6 and 2.0 mg/kg/d dose groups, and significantly increased serum glucose in the 2.0 mg/kg/d dose group. Although T4 (free and total) appeared to be decreased when measured by RIA, free T4 was normal across all dose groups (dams) when measured using equilibrium dialysis. T3 was low at 1.2 mg/kg/d and above in dams but not fetuses and pups. TSH was not elevated and thyroid histology was normal. Dams in the 0.8 mg/kg/d and higher dose groups showed an increase in relative liver weight, a decrease in body weight gain, a decrease in feed consumption, and a slight decrease in the duration of gestation. Pup growth was decreased at 1.2 mg/kg/d and higher. Viability indices for pups from the 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg/d groups were 97, 98, 93, 89, and 82, 49, and 17 percent, respectively.

121 PERFLUOROOCTANESULFONATE-INDUCED PERINATAL MORTALITY IN RAT PUPS IS NOT A RESULT OF REDUCED SERUM LIPIDS.

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This study was designed to test the hypothesis that hypolipidemia causes perfluorooctanesulfonate (PFOS)-induced perinatal mortality in rats. Female rats were supplemented with either 500 mg/kg of mevalonic acid lactone (MAL) twice daily or 500 mg/kg cholesterol (CHOL) once daily and compared to non-supplemented controls. Dose groups consisted of Tween 80 vehicle control, CHOL/vehicle control, MAL/vehicle control, 1.6 mg/kg/d PFOS, 1.6 mg/kg/d PFOS + MAL, 1.6 mg/kg/d PFOS + CHOL, 2.0 mg/kg/d PFOS, 2.0 mg/kg/d PFOS + CHOL, and 2.0 mg/kg/d PFOS + MAL. Female rats were dosed for 42 days prior to mating to untreated males, during mating and through the end of gestation or four days of lactation. Fetuses from eight litters per dose group were taken by cesarean section on day 21 of gestation. All remaining dams were allowed to litter, and were sacrificed with litters on day 5 of lactation. For all or some of the groups, PFOS sera levels and serum lipids, glucose, and mevalonic acid were measured at both time points. For fetuses and gestation day 21 dams, all clinical chemistry parameters remained at or above control levels with the exception of triglycerides (TRIG), which were decreased in fetuses in the MAL-supplemented 2 mg/kg dose group. On lactation day 5, dams had decreased CHOL in all PFOS-treated groups and decreased TRIG in the PFOS-only and CHOL-supplemented PFOS groups. Pups in the CHOL-supplemented 2 mg/kg/d group had decreased TRIG. MAL or CHOL supplementation was unable to prevent or mitigate the perinatal mortality. Viability indices for the Tween 80 vehicle control, CHOL/vehicle control, MAL/vehicle control, 1.6 mg/kg/d PFOS, 1.6 mg/kg/d PFOS + MAL, 1.6 mg/kg/d PFOS + CHOL, 2.0 mg/kg/d PFOS, 2.0 mg/kg/d PFOS + MAL, and 2.0 mg/kg/d PFOS + CHOL were 97, 99, 98, 49, 41, 42, 17, 1, and 14 percent, respectively. Results suggest that the observed perinatal mortality is not a result of hypolipidemia in the perinatal period.

122 CADMIUM ABSORPTION AND ITS RELATIONSHIP TO DIVALENT METAL TRANSPORTER-1 IN THE PREGNANT RAT.

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In pregnancy, the maternal gastrointestinal tract undergoes many physiological and biochemical changes to accommodate the increased demand for essential nutrient absorption and transfer to the fetus. Increased absorption of nonessential metals such as cadmium (Cd) has been reported in pregnant mice in comparison to non-pregnant mice, although the mechanism is not understood. Divalent metal transporter-1 (DMT-1) is localized on the apical membrane of the intestinal epithelial cell and has broad substrate specificity for many divalent metals including Cd. The purpose of this work was to determine whether there is increased Cd absorption in pregnant compared to non-pregnant rats, and whether this correlates with up-regulation of DMT-1 expression. Timed pregnant and non-pregnant female Sprague-Dawley rats were administered 0.4 µmol $CdCl_2$in 4 ml saline/kg (labeled with ^{109}Cd) by oral gavage on gestation day (gd)19. Tissues were collected on gd 20, weighed and assessed for ^{109}Cd content (values expressed as pmol Cd/tissue). There was greater accumulation of ^{109}Cd in duodenum than in jejunem and ileum in both pregnant and non-pregnant rats. However, the amount of Cd in all three portions of the small intestine was higher in pregnant than non-pregnant rats (duodenum 1074 *vs.* 413; jejunem 212 vs.96; ileum 244 vs.107). In addition, more Cd accumulated in the liver and kidney of pregnant than non-pregnant rats (567 *vs.* 249 and 81 *vs.* 40), respectively. The time course of duodenal DMT-1 mRNA expression was determined in pregnant rats by the branched DNA signal amplification method. DMT-1 mRNA levels were about 6-fold higher in duodenum of pregnant than non-pregnant rats. Duodenal DMT-1 mRNA levels in pregnant rats increased from gd 15 through gd 21, as compared to the non-pregnant rats. The correlation between Cd absorption and DMT-1 expression observed during pregnancy suggests the importance of DMT-1 in the absorption of Cd in pregnant rats. (Supported by NIH ES-01142 and ES-07079)

123 REGION SELECTIVE CHANGES OF N-CADHERIN EXPRESSION FOLLOWING *IN OVA* AND *IN VITRO* METHYLMERCURY EXPOSURE TO DEVELOPING CHICK BRAIN.

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Methylmercury (MeHg) exposure during embryogenesis disturbs brain development and results in decreased neurological potential. The mechanism(s) underlying these effects remain unclear. Accumulating evidence suggests that perturbation in the expression of neural adhesion molecules, several families of cell surface proteins which facilitate cellular migration and cytodifferentiation, contribute to brain dysmorphogenesis. Prominent in brain development is N-cadherin, an adhesion molecule whose developmentally-regulated expression is required for proper formation and topographic patterning of the central nervous system. To determine whether changes in N-cadherin expression occur in chick brain following developmental MeHg exposure, embryonic Day 11 chicken eggs were injected with concentrations of MeHg ranging from 0.2 to 2.0 µM and killed 48 hours later. In parallel studies, cortical and cerebellar cultures were prepared from embryonic D11 chicks, exposed to 0.2 to 2.0 µM MeHg and harvested 48 hours after treatment. Protein and RNA were extracted from cerebellar and cortical samples and analyzed for N-cadherin expression using Western blotting and RT-PCR, respectively. Semiquantitative PCR analysis revealed significant reductions in N-cadherin in both *in ova* and *in vitro* exposed chick cerebellum mRNA 48 hours post-treatment, as compared to controls. In contrast, cortical mRNA was not significantly altered in *in ova* or *in vitro*. Western blotting for N-cadherin protein in brain tissue regions was in agreement with mRNA data. The results indicate that MeHg can alter N-cadherin levels in a brain region-selective manner. Disruption of cadherin-dependent morphogenetic events may contribute to the neuroanatomical and neurological defects observed following developmental metal neurotoxicity. Supported by Wilkes University Type I and II research grants.

124 EFFECTS OF RETINOIC ACID ON CELL DEATH DURING *IN VITRO* CHONDROGENESIS.

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Programmed cell death occurs normally in developing limbs helping to give shape and form to the digits and limb. Retinoic acid has been shown through histochemical staining and whole animal studies to affect chondrogenesis and cell death. This study examines the effects of retinoic acid on *in vitro* chondrogenesis and whether this effect is mediated by cell death of chondrocytes. Mesodermal cells from stage 25 embryonic chick limb buds were grown in micromass culture for 5 days. To determine overall effect on chondrogenesis, cultures were continuously exposed to retinoic acid concentrations ranging from 10^{-9} M to 10^{-6} M. Proteoglycan content, an indicator of chondrogenesis, was determined by fixing the cultures, staining the cells with alcian green and visualizing spectrophotometrically at 600 nm. To examine cell death, mesodermal cells from embryonic chick limb buds were grown in micromass tissue cultures and exposed to 10^{-6} M retinoic acid for 48 h beginning on day 5 and day 7 of culture. At the end of the culture period, supernatants were removed from the cultures and lactate dehydrogenase (LDH), an indicator of cell death, was measured. To determine whether cell death was a result of apoptosis, cell cultures were lysed and DNA was observed on 1.5% agarose gel for apoptotic DNA fragmentation. Retinoic acid treatment for the five-day period decreased proteoglycan content as shown by alcian green staining (49.8%, 39.3%, 28.6%, and 15.6% of vehicle control). Levels of cell death as assessed by LDH were increased in treated cultures compared to control cultures (119.3% of control at day 7 treatment). Gel electrophoresis shows that apoptotic DNA fragmentation occurs at earlier times (7 day and 9 day *versus* 14 day) when cultures are treated with retinoic acid. These results suggest that exogenous retinoic acid affects *in vitro* chondrogenesis by increasing inappropriate apoptotic cell death of chondrocytes. (Supported in part by Hatch Funds to MAS and the Interdisciplinary Toxicology Program, UGA).

125 INHIBITION OF ANGIOGENESIS BY 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) IN CULTURED EMBRYONIC HEARTS.

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It has been documented that TCDD reduces coronary angiogenesis on incubation day 10 in chick embryo hearts *in vivo*. In this study we investigated effects of TCDD on coronary vasculogenesis and angiogenesis at an earlier time point of development. Ventricles from 6-day-old control and TCDD-treated (0.3 pmol TCDD/g) embryonic chick hearts were cultured on a three-dimensional collagen gel. After 2 days of growth in normal medium and 3 days of starvation in low serum medium (0.5% fetal bovine serum), the heart explants were fixed, washed and blocked. Endothelial cells, which migrated out from the heart explants and form tubes, were identified by wheat germ agglutinin (WGA) antibody using immunohistochemistry. Three-dimensional images of tubes growing into the collagen gel were obtained by using confocal microscopy. Tube formation also was observed by electron microscopy (EM). Total length of the tubes was measured as a function of the explant's perimeter with use of Image-Pro Plus software. Confocal and EM images showed that the cells growing outward from the myocardial explants formed tubes in culture. Immunohistochemistry revealed that the cells which formed tubes in culture were endothelial in origin, based on the positive WGA staining. Vascular growth, as measured by total tube length divided by explant perimeter, was signifi-

cantly reduced in explants from TCDD-treated chick embryos as compare to explants derived from control embryos. These data suggest that TCDD may inhibit coronary angiogenesis *in vivo* by affecting vascular precursor cells present earlier in cardiac development. Future studies will use this explant culture system to elucidate the function of hypoxia in coronary angiogenesis and the relationship between hypoxia and TCDD in this event. Supported by NIH grant ES-09804.

126 MATERNAL EXPOSURE TO A MIXTURE OF POLYCHLORINATED BIPHENYLS (AROCLOR 1254) EXERTS THYROID HORMONE-LIKE EFFECTS ON NOTCH SIGNALING IN THE FETAL CORTEX.

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Polychlorinated biphenyls (PCBs) are known to be neurotoxic in experimental animals and are associated with neurological deficits in humans. In addition, PCBs are known to reduce circulating levels of thyroid hormones in experimental animals and in humans are associated with measures of thyroid hormones. These observations have led to the hypothesis that PCBs exert at least some neurotoxic effects by reducing circulating levels of thyroid hormones during neural development. Because our Lab. has found that thyroid hormone of maternal origin can affect Notch signaling in the fetal rat brain, we tested the hypothesis that maternal exposure to PCBs can affect Notch signaling in the fetal rat brain. We used HES-1 and HES-5 mRNA expression as markers of Notch activity. Pregnant rats were given Aroclor 1254 (A1254) on a biscuit once daily from gestational day (G) 2 through G15. The doses included 0, 1, and 4 mg/kg. Fetuses were removed on G16 and prepared for measurement of HES-1 and HES-5 mRNA using *in situ* hybridization. We found that A1254 produced a dose-dependent increase in HES-1 expression in the ventricular zone of the fetal cortex, but did not significantly affect HES-5 expression. Moreover, these effects were observed in the same region of the cortex that was found to be affected by manipulating maternal thyroid status. Because HES expression is associated with differentiation of radial glia from neural precursor cells in the ventricular zone of the fetal cortex, these results suggest that PCB exposure may result in a shift toward the production of more glia and fewer neurons in the early cortex. Studies are under way to test this hypothesis and to determine whether effects persist into adulthood. Supported by ES10026 and EPA STAR Graduate Fellowship.

127 PCB-INDUCED CHANGES IN THE DNA-BINDING OF SEVERAL TRANSCRIPTION FACTORS IN THE DEVELOPING CEREBELLUM.

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Polychlorinated biphenyls (PCBs) are a class of persistent chemical pollutants prevalent in the environment despite the ban of their use for decades. Disturbances in brain development and cognition are among the neurotoxic manifestations of PCBs. The cellular and molecular basis for PCB-induced developmental neurotoxicity is still unclear, however, a series of *in vitro* and some *in vivo* studies have revealed that the disruption of Ca^{2+} homeostasis and Ca^{2+} mediated signal transduction play a significant role. The culminating event in a variety of signal transduction pathways is the regulation of gene expression. Therefore, we examined the DNA-binding of several transcription factors in order to identify those that are involved in signal transduction/transcription coupling in the developing brains of PCB-exposed animals. Pregnant rats (Long Evans) were exposed perinatally to 0 or 6 mg/kg/day of Aroclor 1254 (AccuStandard Inc., Lot # 124-191) from gestation day 6 through postnatal day (PND) 21. On specific PNDs viz., 7, 14, 21, and 60, the cerebellum was isolated. The DNA-binding of various transcription factors: Sp1, AP1, NF-kB and CREB was then monitored using gel mobility shift assays. While an overall transient increase in the DNA-binding of all the transcription factors was observed following birth, this period was characterized by a pronounced induction in Sp1 and NF-kB DNA-binding. The distinct changes in Sp1 and NF-kB DNA-binding suggest that they may be involved in mediating PCB-induced perturbations in gene expression that occur during perinatal brain development. (The abstract does not necessarily reflect USEPA policy).

128 DO POLYCHLORINATED BIPHENOLS INTERFERE WITH NON-GENOMIC ACTIONS OF THYROID HORMONES?

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Polychlorinated Biphenols (PCBs) are known neurotoxins. It has been suggested that PCB neurotoxicity is mediated in part by disruption of thyroid hormone action on its receptor. However, individual PCB congeners have not been shown to interact avidly with the thyroid hormone receptor. In contrast, individual PCB congeners have been shown to compete with thyroxine for binding to various blood and cellular proteins. Because specific non-genomic actions of thyroid hormone are mediated by T4, not T3, and are not mediated by the T3 receptor, we proposed that specific PCBs may affect the ability of T4 to exert these non-genomic actions. It has been reported that T4 promotes the formation of F (filamentous)-actin fibers in neonatal rat astrocyte cultures. This process is important for contact-mediated cell signaling and may mediate the effect of thyroid hormone on specific developmental events. Therefore, the goal of these studies was to test whether PCBs interfere with the T4 mediated formation of F-actin fibers in this tissue culture system. The original experimental design was to develop a simple and quantitative assay for the amount of F- and G- actin using dot blots and a standard curve derived from known amounts of purified actin. While we were successful in establishing a standard curve for the purified actin, the F-actin samples were not sufficiently soluble for use with dot blots. We are currently in the process of validating western blots to quantify actin - a requirement for testing this hypothesis. The larger significance of this work is two-fold. If we document that individual PCB congeners affect T4-mediated F-actin polymerization, it would represent a potentially important mechanism by which PCBs mediate their neurodevelopmental effects. Additionally, it would likely indicate that different congeners may exert independent effects on different elements of the thyroid system.

129 *IN UTERO* TOXICITY OF COPLANAR HEXABROMOBIPHENYL ASSOCIATED WITH PRESENCE OF THE AROMATIC HYDROCARBON RECEPTOR.

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Toxicity of polybrominated biphenyls (PBBs) first became evident following the 1973 Michigan disaster in which about 1, 000 lbs of Firemaster was inadvertently added to cattle feed. Cows, chickens and pigs became sick, and thousands of farm animals were destroyed. Humans eating dairy products also manifested neurological symptoms, including a possible association with lowered I.Q. in children who were *in utero* at the time. Neonatal lethality from maternal PBB exposure has also been reported in the offspring of mink fed diets containing Firemaster. We have chosen to assess *in utero* toxicity in pregnant C57BL/6J (B6) mice (having the high-affinity aryl hydrocarbon receptor (AHR) and B6.D2-Ahrd congenic mice (having the poor-affinity AHR on a B6 background), treated at gestational day (GD)5 with 100 mg/kg coplanar 3, 4, 5, 3', 4', 5'-hexabromobiphenyl (cHBB) or non-coplanar 2, 4, 6, 2', 4', 6'-HBB (ncHBB). In controls, ncHBB-treated B6 and B6.D2-Ahrd, and cHBB-treated B6.D2-Ahrd, 100% of offspring survived birth and the weanling period. In cHBB-treated B6 mice, however, 100% of the pups died within the first 1-5 days post partum. Histologic exam of the B6 pups showed lesions in the liver with white-cell infiltrates, indicative of hepatic failure, whereas the mother's liver appeared normal. Northern blot and RT-PCR analysis confirmed CYP1A1 mRNA induction in fetal liver and brain at GD18 in cHBB-treated B6 but no other groups. The mechanism of neonatal toxicity remains unknown, but would appear to be AHR-mediated. Lower doses of cHBB and behavioral tests in surviving pups at 60 days of age are planned. ——Supported, in part, by NIH grants P30 ES06096 and R01 ES08147.

130 ANTISENSE EVIDENCE FOR NF-κB-MEDIATED SIGNAL TRANSDUCTION IN THE MECHANISM OF PHENYTOIN EMBRYOPATHIES.

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The nuclear transcription factor-kappa B (NF-κB) family regulates expression of many genes, some of which have developmental roles. Excessive NF-κB activation is implicated in the pathogenesis of several diseases including cancer. NF-κB is activated by numerous stimuli, many of which increase the levels of reactive oxygen species (ROS), and activation is inhibited by some antioxidants. NF-κB may also be a downstream effector of ROS-mediated signal transduction, such as the Ras pathway. Teratogens like the anticonvulsant drug phenytoin enhance embryonic ROS formation, and embryopathies may result from oxidative damage to cellular macromolecules, and/or enhanced ROS-mediated signaling. We previously showed that phenytoin increases embryonic Ras activation, and Ras inhibition blocks phenytoin embryotoxicity. To determine the toxicological role of ROS-mediated Ras activation of NF-κB signaling, embryos were cultured with either a therapeutic concentration of phenytoin (20 μg/ml, 80 μM) or its vehicle (0.002N NaOH), with or without antisense NF-κB (p65) (2.5 - 25 μM). Gestational day 9.5 CD-1 embryos incubated with phenytoin showed decreases in anterior neuropore closure, turning, yolk sac diameter, crown rump length and somite development ($p<0.05$). Addition of antisense NF-κB to the culture medium decreased the embryotoxic effects of phenytoin on anterior neuropore closure, turning and somite development

(p<0.05), but did not protect against decreases in yolk sac diameter and crown rump length. The protective effects were not observed using sense or nonsense controls, or antisense vehicle, suggesting that NF-κB-mediated signal transduction may play a role in the mechanism of phenytoin embryopathies. (Support: Canadian Institutes of Health Research)

131 p53 MODULATION OF PHENYTOIN EMBRYOPATHIES IN p53 KNOCKOUT MICE.

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The tumour suppressor protein p53 facilitates DNA repair and protects against the embryopathic effects of DNA-damaging drugs like BENZO(A)PYRENE, which enhances embryonic reactive oxygen species (ROS) formation. We previously found that p53 protected against fetal resorptions and postpartum lethality (PPL), caused by the ROS-initiating anticonvulsant drug phenytoin given on gestational days (GD) 12 and 13 (plug=GD 1). Since p53 expression declines during embryogenesis, we determined here whether its modulation of phenytoin embryopathies differed when phenytoin was given earlier in gestation. Virgin female heterozygous (+/-) p53 knockout mice were mated with p53+/- males, dams were administered phenytoin (65 mg/kg ip) or its saline vehicle on GDs 10 and 11 at 0900 hr, and resorptions and fetuses were characterized on GD 19. Compared to vehicle controls, phenytoin caused fetal body weight loss similarly in all fetal genotypes, and enhanced PPL in wild-type (+/+) and +/- fetuses (p<0.05). In phenytoin-exposed fetuses, there was a reciprocal p53 gene dose-dependent increase in fetal resorptions in +/+ (8%), +/- (18%) and -/- (23%) fetuses, although this trend was not significant. Conversely, loss of p53 alleles resulted in a gene dose-dependent decrease in PPL in +/+ (78%), +/- (48%) and -/- (30%) fetuses (+/+ *vs.* +/-, p=0.016; +/+ *vs.* -/-, p=0.008). The GD 10/11 p53-dependent resorption protection was similar to results with GD 12/13 phenytoin treatment, while the PPL enhancement by p53 was opposite. These results show that p53 differentially modulates phenytoin embryopathies in a gestationally dependent fashion, suggesting different developmental mechanisms. (Support: Canadian Institutes of Health Research)

132 MICROARRAY ANALYSIS OF DEVELOPMENTAL TOXICITY: ONTOGENETIC PROFILES OF SUSCEPTIBILITY IN THE MOUSE EMBRYONIC EYE.

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The developing mouse eye is sensitive to many chemical teratogens. Microphthalmia, for example, occurs with intrauterine exposure to 2-chlorodeoxyadenosine (2CdA, D8 of gestation) or methylmercury (MeHg, D9 of gestation). Stage dependent differences in the responsiveness of the developing eye to different agents implies serial changes in genetic susceptibility during development. The purpose of this research is to profile these changes, classify them with respect to developmental patterns and biomolecular functions, and finally to analyze their behavior during critical periods following exposure to ocular teratogens. CD-1 mouse embryos, staged by somite number, were microdissected to enrich for optic pit (D8), optic vessicle (D9), and optic cup (D10). RNA isolated and labeled with fluorescein or biotin nucleotides was used for competitive fluorescent hybridization to microarray probes followed by tyramide signal amplification. Replicate analysis with dye-swapping was done for each sample. Normalized expression ratios were subjected to hierarchical clustering using a similarity metric based on standard Pearson correlation and distances computed by average linkage clustering. Genes determined to comprise the developmental core response represented approximately 3-5% of all those arrayed. At least ½ exhibited a patterned response behavior with the ocular teratogens. Potential signaling/regulatory genes that increased with eye development decreased with MeHg. Others involved in intermediate metabolism, including the lower glycolytic block, decreased with both development and MeHg. These findings may provide potential insight to the mechanism of ocular teratogenesis by MeHg and other similarly acting agents, and in doing so, elucidate measures of intervention. (Supported by grants T32 ES07282 and RO1 ES 09120 from NIH/NIEHS and STAR G9B1022 from the USEPA, but does not reflect USEPA policy)

133 ALCOHOL DEHYDROGENASE ACTIVITY FROM ZEBRAFISH LIVER AND RECOMBINANT ZEBRAFISH ADH3 PROTEIN.

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In continuation of our studies with ethanol toxicity and developmental expression of alcohol dehydrogenase (ADH) mRNA in zebrafish embryos the following studies were undertaken to identify cytosolic alcohol dehydrogenase of adult zebrafish liver that metabolize alcohols to their corresponding aldehydes. Oligonucleotide primers were used to amplify the entire coding region of ADH3 cDNA by reverse transcriptase polymerase chain reaction using total RNA prepared from adult zebrafish AB strain liver. Complete cDNA encoded a 376 amino acid polypeptide that exhibits 90% sequence identity to ADH3 from the marine fish *Sparus aurata* and 82 and 81% identity to the mouse and human sequences, respectively. Zebrafish strains show differential sensitivity to developmental alcohol exposure, and embryos also show differential expression of the ADH3 mRNA. We have expressed zebrafish ADH3, with molecular mass of 40 kDa, in *E.coli* for analysis of enzyme activity toward ethanol, hexadienal, acetaldehyde, lactic acid, and other substrates. We have also initiated the production of polyclonal antibodies against recombinant zebrafish ADH3 for immunohistochemistry and expression studies. The results will be used in conjunction with mRNA expression data and *in situ* hybridization analysis to determine the role of ADH3 in the developmental effects of ethanol exposure in zebrafish. Supported primarily by the National Institute on Alcohol Abuse and Alcoholism (NIH R03 AA12408), the University of Wisconsin-Milwaukee, UWM Marine and Freshwater Biomedical Science Center (NIH P30 ES04184) and the Medical College of Wisconsin.

134 IDENTIFICATION AND CHARACTERIZATION OF ALCOHOL DEHYRDOGENASE III (ADHIII) IN ZEBRAFISH.

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Ethanol has been shown to be a teratogen in experimental animals and in humans. The molecular mechanism of ethanol-mediated fetal injury remains unclear. There are two proposed mechanisms of action to explain the teratogenic effects of ethanol. The first mechanism is related to ethanol's ability to directly affect development and the second requires ethanol metabolism. The focus of this work is on the indirect, or metabolism dependent, processes. We are using zebrafish as a model because they share many cellular and physiological characteristics with higher vertebrates including humans and offer many practical advantages. For example, the embryos develop externally, removing the complication of maternal/placental/fetal interactions, and are transparent so any developmental stage can be observed without interfering with normal embryogenesis. The goal of these studies is to determine if there is an association between ethanol metabolism and ethanol-mediated developmental toxicity. Here we report the isolation and characterization of a cDNA encoding a zebrafish alcohol dehydrogenase (zfADH). The 1450 bp zfADH is 78%, 72%, and 77% similar to the cod fish ADH, the human ADHIII (glutathione-dependent formaldehyde dehydrogenase), and to another zebrafish ADHIII clone (GenBank Accession AF399909) at the amino acid level, respectively. To confirm that the zfADHIII has the ability to metabolize ethanol, the protein was overexpressed in baculovirus infected SF-9 insect cells. For functional studies, the zfADHIII protein was purified to homogeneity by 5'-AMP agarose affinity chromatography. Initial kinetics indicated that this ADHIII metabolizes ethanol and a variety of other substrates. Since this protein has the ability to metabolize ethanol, *in situ* hybridization experiments were conducted to determine the temporal and spatial expression of zfADHII during zebrafish development. These studies indicate that zebrafish is a suitable model to investigate ethanol-mediated developmental toxicity. This research was supported by NIH/NIAAA grant #AA12783.

135 GESTATIONAL AND LACTATIONAL EXPOSURE TO ESTROGENIC CHEMICALS DOES NOT AFFECT MAMMARY GLAND DEVELOPMENT IN FEMALE MICE.

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There exist paradoxical results demonstrating opposite effects of prenatal and postnatal exposure to estrogens on mammary gland development, however, the effects of gestational and lactational exposure to estrogens has not been described. The objective of the study was to assess the effects on mammary gland morphology following gestational and lactational exposure to maternal doses of 0, 0.1, 1, and 10 µg/kg diethylstilbestrol (DES) or 0, 0.1, 0.5, 2.5 and 10 mg/kg genistein (GEN) from gestational day 12 to postnatal (PND) 21. The doses of genistein approximated human exposure levels. Body weight and anogenital distance (AGD) of female B6D2F1 offspring were measured on PND 7, 21 and 49. Mammary gland whole mounts were examined on PND 49 for epithelial growth (% of fat pad occupied by epithelium), length of mammary epithelial tree, number of terminal end buds (TEBs, undifferentiated proliferating structures) and density of alveolar buds (ABs, differentiated milk secreting lobules). Prior to analyzing female offspring, an initial study was performed to determine the time course (3, 4, 5, 7 and 10 weeks)

of mammary gland development in naïve female B6D2F1 mice. Seven weeks of age was chosen as a suitable time point to examine treatment-related changes in mammary development. There were no significant changes in body weight or AGD in female offspring of DES or GEN treated dams. Although there was a trend towards an increase in mammary gland growth and an increase in alveolar bud formation in DES-exposed offspring, the results were not significant due to large interlitter variability. We also did not detect any significant changes in mammary growth, TEBs, or ABs in GEN-exposed offspring. These results suggest that combined gestational and lactational exposure to genistein has little effect on mammary gland morphology at human exposure levels.

136 EFFECT OF *IN UTERO* AND LACTATIONAL EXPOSURE OF MICE TO RECOMBINANT HUMAN INTERLEUKIN-10.

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Pregnant mice received daily s.c. doses of 0, 2, 4 and 8 mpk rhuIL-10 from gestation day 6 through postpartum day (PPD) 21 to investigate the effect of rhuIL-10 on fetotoxicity, offspring development, and the survival and reproductive capabilities of an F1 generation. Dam mortality (pregnant and non-pregnant) during the dosing period was 3, 9, 12 and 6% in the control, 2, 4 and 8 mpk dose groups, respectively. The deaths were not associated with abnormal clinical signs, body weights, food consumption or necropsy findings, and the majority occurred after 14 doses which, for the pregnant mice, coincided with the day of parturition. However, the timing also coincided with deaths in non-pregnant mice and male mice in other studies, which suggested that the deaths were unrelated to parturition. Although non-dose-related, the deaths were considered related to rhuIL-10; a similar pattern of mortality was seen in other studies in which the deaths coincided with the development of anti-IL-10 antibodies. Litter sizes of 11.5, 11.4, 10.1 and 9.9 liveborn pups/litter and pup mortality for PPD 0-4 of 6, 5, 18 and 17% were found in the control, 2, 4 and 8 mpk groups, respectively (mortality for PPD 5-21 was unaffected). The pup deaths (found dead / cannibalized) were not associated with abnormal clinical signs, gestation length, parturition or necropsy findings. F1 pups developed normally to sexual maturity with no effects on reproductive fertility to produce F2 pups with normal litter size, birth weight and survival. It is concluded that a combination of slightly increased resorptions (not measured but seen in a previous study), an increased number of "found deads" and possibly unobserved cannibalizations (during or immediately after parturition) contributed to the increased F1 pup mortality / litter size at birth.

137 RAPID RISE OF BLOOD LEAD IN PATIENTS WITH RETAINED LEAD BULLETS.

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Lead exposure in gunshot victims with retained lead bullets has been poorly studied, despite more than a century of published case reports and over 50,000 new cases each year in the US alone. We studied a series of nearly 300 such patients to determine the time course of blood lead concentration (PbB) for up to six months after injury and to determine the risk factors for elevated PbB. All patients in the study had retained bullets verified by x-ray. We took blood to determine PbB at the time of informed consent (mean=3.2 days after injury), again when we measured bone lead concentration (PbBone) (mean=22.4 days), at 90 and 180 days after injury. Geometric mean (range) PbB at admission, 3, 22, 90, and 180 days after admission was 2.0 (0.3-11.8), 3.4 (0.2-23.0), 5.8 (0.3-30.2), 7.3 (0.8-38.6), and 5.5 (1.7-28.4) µg/dL, respectively. Mean (range) tibia and calcaneus PbBone was 5.5 (-16.9 - 26.2) and 9.5 (-22.0 - 39.7) µg/g. Although only one patient exceeded 10 µg/dL upon admission, 10.2% and 1.8% of patients exceeded 10 and 20 µg/dL at 3 days, 28.4% and 6.8% exceeded these limits at 22 days, 38.3% and 14.8% at 90 days, and 28.9 and 7.9% at 180 days. Repeated measures analyses up to 22 days after injury revealed that PbB of patients with bone fracture increased faster and achieved higher PbB than other patients. The odds ratio of patients with bone fracture exceeding 20 µg/dL during the first 22 days was 8.9 (95% confidence interval 1.7-46.0). Over 22 days, patients with higher calcaneus PbBone and patients over 60 years had higher risk of exceeding 20 µg/dL. Continued surveillance of these patients will reveal the long term time course of PbB and the factors predicting patients who will exceed national and international norms.

138 TRABECULAR BONE LEAD CONCENTRATION INFLUENCES BLOOD PRESSURE DURING PREGNANCY IN IMMIGRANT WOMEN.

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To determine the relationship between past (bone lead; PbBone) and present (blood lead; PbB) lead exposure on blood pressure (BP) during pregnancy we analyzed prenatal (3rd trimester) and postnatal (mean≅68 days after birth) PbB and BP from over 600 immigrant women, 99% from Latin America. Geometric mean (range) prenatal and postnatal PbB was 2.0 µg/dL (0.4-38.9) and 2.4 µg/dL (0.4-51.4). Postnatal mean (range) tibia (cortical bone) and calcaneus (trabecular) PbBone (measured by *in vivo* K-shell x-ray fluorescence) were 8.6 µg/g (-33.6 - 62.2) and ll.3 µg/g (-30.1 - 66.4). Mean (range) prenatal and postnatal systolic and diastolic BP was 108.4 mmHg (84-138) and 59.4 mmHg (36-80), and 109.3 (82-136) and 69.7 (33-84). We used structural equation modeling to simultaneously account for the effects of age, body mass index, PbB, and PbBone on BP during and after pregnancy. The model indicated that of all lead measures only calcaneus PbBone was associated with BP during pregnancy, and no bone lead measure accounted for maternal BP postpartum. After controlling for the other factors influencing prenatal blood pressure, every increase of 10 µg/g in calcaneus PbBone is associated with a 0.5 mmHg increase in diastolic blood pressure. The PbBone-BP relationship in normotensive pregnant women is similar to that found between trabecular PbBone and risk for hypertension in aging women in published work from another laboratory. While the effect is small, it indicates that populations with a wide range of past lead exposure may be at increased risk for elevated blood pressure or hypertension, regardless of present exposure.

139 ASSOCIATION OF δ-AMINOLEVULINIC ACID DEHYDRATASE GENOTYPE WITH BLOOD LEAD AND ZINC PROTOPORPHYRIN LEVELS IN A MEXICAN POPULATION ENVIRONMENTALLY EXPOSED TO LEAD.

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It is known that there are interindividual variations in the susceptibility to lead (Pb) toxicity. Evidence suggests that the polymorphism at certain genetic loci is known to be related to differentially inherited responses to Pb. Two polymorphic genes have been studied, the vitamin D receptor gene and the δ-aminolevulinic acid dehydratase (ALAD) gene. The polymorphism of ALAD gene ($ALAD^1$ and $ALAD^2$) has been associated with differential accumulation and distribution of Pb in blood, bone, and internal organs. Subjects who have at least one copy of the $ALAD^2$ allele have higher blood Pb (PbB) and lower zinc protoporphyrin (ZPP) levels; however, most of these studies have been done in subjects with high PbB. We investigated the potential impact of ALAD genotype on Pb toxicokinetic (PbB and ZPP) in a Mexican adult population from Northern Mexico environmentally exposed to Pb. We conducted a cross-sectional study in 191 adults (both sexes) randomly selected. The ALAD genotyping was based on the PCR method, PbB were determined by voltametry and ZPP by hematoflurometry. The $ALAD^2$ allele frequency in this Mexican population was 5.2% (0.5% $ALAD^2$ homozygote), relatively lower than the frequency reported in an infant hispanic population (Astrin et al. 1987. Ann NY Acad Sciences; 514:23-29), although this study had a selection bias. The average PbB was 9.3µg/dl, with a range from 1.2 to 37µg/dl. We found no significant differences among participants according to ALAD genotype with respect to PbB (p=0.244) or ZPP (p=0.123), even after controling by potential confounders. These preliminary results suggest that ALAD genotype may have no influence on Pb kinetics and toxicity in adults at low Pb-exposure levels. This study was supported by CONACyT-México (Grant 31024-M).

140 SPERM CHROMATIN STRUCTURE IN MEN ENVIRONMENTALLY EXPOSED TO LEAD.

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The evaluation of sperm chromatin is relevant since chromatin abnormalities may be associated with reproductive failures and offspring development. Despite the condensed structure of human sperm chromatin, it can be damaged by exposure to

toxic agents. Lead (Pb) exposure is still a public health concern in developing countries, which detrimental effects on male reproduction are not well studied. Few studies have reported Pb effects on sperm chromatin; humans occupationally Pb-exposed have shown a decreased sperm chromatin condensation, but little is known about Pb effects at low exposure levels. Therefore, we evaluated sperm chromatin in 66 semen samples from men environmentally exposed to Pb (blood Pb levels of 10.3µg/dl) by the SDS-decondensation technique (SDS-ndc) and the sperm chromatin structure assay (SCSA) by flow cytometry. Results were correlated with Pb and zinc (Zn) levels in semen compartments determined by atomic absorption spectroscopy. SDS-ncd showed that chromatin condensation significantly increased as Zn in spermatozoa (ZnSpz), in seminal liquid (ZnLS), and Pb in seminal liquid (PbLS) increased. From the SCSA parameters, only green fluorescence (GRN, accessibility to dyes) negatively correlated with SDS-dnc. Alpha-t positively correlated with ZnSpz and ZnLS, whereas Pb did not. Comp-αt (DNA denaturation) did not correlate with Zn nor Pb in any compartment. GRN negatively correlated with ZnSpz and ZnLS ($p<0.05$), but not with PbLS ($p=0.144$). In addition, positive correlations were observed between ZnSpz and ZnLS with PbLS. Taken together, these results suggest that the increase in chromatin condensation observed with Zn may be an indirect effect of Pb. Thus, these results support that the exposure to low Pb levels alters the sperm chromatin integrity, but does not damage sperm DNA. Further studies in larger populations are needed to confirm the indirect effect of Pb. This study was supported by CONACyT-Mexico (Grant 31024-M).

141 TOXIC EFFECTS OF LEAD ON OCCUPATIONALLY EXPOSED CHILDREN.

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53 Children in the age group of 9 - 15, from different occupational environments and working as petrol sniffers, printing press workers, motor mechanics and working in petty shops whose shops are stationed at traffic Junctions, were chosen in this study. Blood samples of the test subjects were assayed for blood lead, total red blood cell(RBC) count, Total white blood cell(WBC) count, differential count, haemoglobin, delta amino levulinic acid dehydratase and haemsynthatase. 16 children showed blood lead more than 70 microgram/100 ml. RBC and haemoglobin significantly decreased. Basophilic stipling was prominently seen and subjects were found anemic with microcytic and hypochromic red cells. Blood lead served highly suitable and dependable indicator reflecting ill health of these children. Depleted levels of delta amino levulinic acid dehydratase and haemsynthatase were found. Genotoxity of lead was found in 15 per cent cases as shown by SCG test. 7 children showed bluish teeth, dermatitis and graying of hairs. An attempt will be made to present and discuss certain markers more specific, more reliable and suitable for assessing toxicity in occupationally lead exposed children.

142 DETERMINATION OF THE ENDOGENOUS LEAD CONTRIBUTION TO BLOOD IN CHILDREN USING A LEAD ISOTOPES APPROACH.

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The accumulation of lead (Pb) in skeletal tissues of children during Pb exposure acts as an internal Pb repository that controls the rate of decrease of blood Pb level after Pb exposure is reduced. Challenges associated with assessing the magnitude of this endogenous Pb input to blood confounds the ability to determine the efficacy of household Pb abatement practices. Here, we are implementing a novel isotopic approach to measure the endogenous release of Pb into blood in children. The Pb isotopic compositions (Pb IC) of blood, and feces (four consecutive daily collections) from children with blood Pb levels between 20 and 35 µg/dL were measured. The Pb content and Pb IC of feces were assumed to represent those of the child's Pb intake. In cases where the Pb IC of blood and the average feces were different, a household abatement was done to reduce the Pb contribution of environmental sources to blood, and to induce a shift in the Pb IC of blood towards the average isotopic composition of the endogenous Pb source(s). Two and six months after intervention, the same sampling protocol is to be repeated to obtain Pb contents and isotopic measurements of the new Pb intake and blood. Through a series of mass balance equations we will then derive the endogenous Pb contribution to blood. Preliminary results of the first round of blood and feces collection from several cases indicates that while in some cases the variability in the Pb IC of feces from consecutive collection days may be quite large, the Pb-content-weighted average isotopic composition of all collected fecal samples was significantly different than the Pb IC of blood. This indicates that in the cases examined the Pb IC of intake and endogenous sources are different, thus meeting a necessary condition required to quantify the endogenous contribution of Pb to blood post-intervention.

143 PYRROLOQUINOLINE QUINONE AS A NOVEL THERAPEUTIC CHELATING AGENT TO REDUCE TISSUE LEAD LEVELS AND TOXICITY.

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Childhood Pb poisoning continues to be a significant public health problem in the US with estimates of nearly 1, 000, 000 affected children. Recent clinical and laboratory studies have suggested that chelation treatment of subjects with blood Pb levels <40µg/dL is not efficacious for alleviating some measures of neurocognitive toxicity. Here we report data on the efficacy of a potentially novel chelating compound, the trianionic N-heteroaromatic molecule pyrroloquinoline quinone (PQQ), for reducing soft tissue Pb levels in a rodent model of human Pb poisoning. A preliminary study was conducted in which PQQ was administered *via* i.p. injection (10mg/kg/day x 5 days). The effects on tissue lead levels were evaluated in comparison to vehicle and succimer (DMSA, 25mg/kg/day x 5 days) treatments. When administered i.p., PQQ treatment significantly reduced blood ($p=0.008$) and kidney ($p<0.000$) Pb levels relative to vehicle treated animals, to an extent comparable to succimer. Brain Pb levels were reduced with both PQQ and DMSA treatment, but only the DMSA treatment reached statistical significance. A larger PQQ study is currently underway with dosing *via* oral gavage (10mg/kg/day x 14 days) to evaluate efficacy through that delivery route. These preliminary data demonstrate that PQQ shows promise as an efficacious chelating agent for the reduction of body Pb levels. These results also warrant further research into the use of PQQ as a treatment for childhood Pb poisoning, and its possible use also as a preventative agent against Pb poisoning.

144 THE RELATIONSHIP BETWEEN LEAD IN PLASMA AND WHOLE BLOOD IN WOMEN.

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Studies have suggested that plasma lead (Pb) levels may better reflect the toxicologically labile fraction of circulatory Pb that is more freely available for exchange with target tissues than do whole blood Pb levels. Here, we conducted a longitudinal assessment of the relationship between Pb in whole blood and plasma in environmentally exposed reproductive age women (n=63) living in Mexico City, Mexico. Samples were collected using trace metal clean techniques and analyzed for Pb using HR-ICP-MS. A subset of subjects provided repeated blood samples weekly for 4 weeks (n=17 subjects), or every 1 to 2 months over 9 months (n=14 subjects). Plasma Pb concentration was significantly positively associated with whole blood Pb in a curvilinear fashion over the range of blood Pb values observed here (2.13 - 39.7 mg/dL). This relationship was best described by the function: Plasma Pb = e(-2.392 + 0.0898 * blood Pb), where SEcoefficient = 0.0054, SEconstant = 0.063 (n=63 subjects, n=141 observations). Results from the short and long-term repeated collection subjects indicated that the within and between subjects variance components were not significantly different between the two subsets of subjects. The between subjects component accounts for 78% of the variance in plasma Pb levels, while the residual variance (22%) maybe attributed to other unmeasured factors. Analysis of the %plasma Pb Vs blood Pb relationship at blood Pb levels <10ug/dL suggests that relationship is neither constant or linear. This study demonstrates that plasma Pb measurements may be applied to general clinical settings, provided that established trace metal clean techniques are adopted. They also show that the relative (%) partitioning of whole blood Pb in plasma naturally varies by a factor of ~2 to 4-fold between subjects at a given blood Pb level. Overall, these data indicate that plasma Pb may provide a more meaningful measure of toxicologically available Pb that may complement whole blood Pb level measurements.

145 AN ICP-MS METHOD FOR THE DETECTION OF DEPLETED URANIUM IN BIOLOGICAL FLUIDS.

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Because of concerns about the potential adverse health effects of exposure to depleted uranium (DU) during combat, the development of a uranium (U) isotope screening method for biological fluids is a pressing need. Current methods to determine U concentration and isotope composition in urine have low precision, and are very laborious and expensive due to the difficulties to measure the very low amounts of uranium found in urine (pg/mL). We have developed an inductively coupled plasma mass spectrometry method to measure U concentration and iso-

topic ratios, with no pre-concentration steps and direct introduction into the plasma. Urines are acidified and spiked with ^{209}Bi to monitor sensitivity changes during the sample runs. The main sources of measurement error are memory effects in the sample introduction system and unsteady blanks of 235U over the course of the run. To minimize these errors, samples were run manually in self-aspiration mode in an order of increasing U concentration determined semi-quantitatively before the run. The quantification limit for U concentration is 3 pg/mL. U isotope ratio precision was evaluated as the relative standard deviation (RSD) of repeated measurements over the course of a year (10 different runs) of three types of solutions: Isotopic measurements of U concentration standards in the range 11.4 pg/mL to 122 pg/mL yielded 6% to 2% RSD (n=9), respectively; isotopic determinations of the U isotopic standard NBSU010 measured at 8.9 pg/mL yielded 3.7% RSD (n= 15), and a low U content urine spiked with NBSU010 to a final U concentration of 21.4 pg/mL had an RSD of 7.9% (n = 10). Accuracy was evaluated from measurements of the U isotopic ratio of NBS U010, which yielded a value of 99.0±0.9 (SE) (n= 15), well within the certified value of 98.6. Application of this technique to Gulf War vets verified the presence of DU in urines from vets with embedded DU shrapnel.

146 CHRONIC DOSE-RESPONSE STUDY OF LEAD TOXICITY IN RATS: ASSIGNMENT OF A LOWEST OBSERVED EFFECT LEVEL IN A DRINKING WATER STUDY.

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Male Sprague-Dawley rats were administered concentrations of 25, 50, or 100 ppm lead (as lead acetate) in deionized drinking water for 30, 90, or 180 days. At the 180 day timepoint, kidney/body weight ratios were 70%, 75%, and 70% that of the control value, and kidney aminolevulinic acid dehydratase (ALAD) levels were 56%, 33%, and 33% that of the control value in the 25, 50 and 100 ppm dose groups. Decreased kidney/body weight ratios at 180 days were attributed to increased terminal body weights, which ranged from 130-138% that of the control group. Significant changes in other variables were observed, but only at the 30 day and 90 day timepoints. Decreases in blood ALAD and increases in urinary protein were observed after 90 days of lead exposure. At 90 days, blood ALAD levels were 59%, 54%, and 57% that of control in the 25, 50, and 100 ppm dose groups. At this same timepoint, urinary protein was 143%, 155%, and 163% that of the control. These data suggest that some adaptation/compensation took place because these changes were not observed at 180 days. Histopathological analyses of kidney sections showed usual morphologic alterations associated with lead exposure. Compared to control animals, a larger number of mitotic figures were observed in proximal tubule cells in test animals in all dose groups after only 30 days of lead exposure. Karyomegaly along with eosinophilic inclusions in enlarged atypical nuclei were observed in proximal tubule cells among 50 and 100 ppm test animals. At 90 days, karyomegaly with inclusion bodies was observed in the 100 ppm group. At 180 days, karyomegaly with inclusion bodies was observed in the 50 ppm and 100 ppm dose groups. Cells with karyomegaly were primarily from the P3 segment. Based on changes observed at all lead concentrations, a LOEL of 25 ppm was assigned. This LOEL is currently being used in a six-month metal/metalloid mixture study. [Supported by USEPA Star Grant R827161-01-0].

147 MANGANESE TOXICITY: A COMPARATIVE REPRODUCTIVE TOXICITY IN RATS AND MICE.

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The present study was designed to determine if manganese exposure caused species specific responses in rodents. CD-1 Mice and Sprague-Dawley rats were investigated for alterations in reproductive parameters after administration of manganese acetate by oral gavage. Reproductive toxicity produced in mice occurred at much lower doses than in rats. The dose-range of 50 mg/kg was determined to be toxic for mice, whereas 2500 mg/kg was toxic to rats. Rats were dosed with a sublethal concentration of 76.5, 153, 306 mgMn/kg for 63 days. Mice were dosed with 7.5, 15 and 30 mg Mn/kg for 43 days. Cross-mating was achieved between the high dose of animals of each sex with controls of opposite sex, and others were mated with their respective groups. After a 5-day cohabitation period with females, the males were separated. On day 18 of gestation, necropsy was performed and a comprehensive examination was made of reproductive organ weights. Other end points observed included uterine implantation sites, resorptions, fetus number, fetus weight and the corpora lutea. Differences in pregnancy rates, fetal weights, fetal number, resorptions and in number of *corpora lutea* were observed between the control negatives, control positives and treated groups of rats and mice. The results of the present study demonstrate that mice are more sensitive to manganese as compared to rats. In both the species, control females mated with high dose males had fewer pregnancy rates, and males were affected more than the females. Our study suggests that the pattern of response was same in both species even though it occurred at different concentrations. As a result, mice and rats may both be a valid model of toxicity testing, even though differences between the rodents exist. Supported by ATSDR #U50/ATU398948-10.

148 INDUCTION OF HEPATIC METALLOTHIONEIN BY MANGANESE IS MEDIATED BY INTERLEUKIN-6.

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Inducers of metallothionein (MT) include metals, cytokines, hormones and other physical and chemical stressors. Induction of MT by metals such as Zn has been shown to be mediated by a specific transcription factor, MTF1. However, the mechanism of MT induction by other transition metals including Mn has been poorly elucidated. Therefore, we investigated the characteristics of Mn-induced MT and the mechanism of MT induction by Mn. Male ICR mice were injected subcutaneously with $MnCl_2$ and sacrificed 24 h after the injection. MT concentration was increased dose-dependently in the liver by $MnCl_2$ administration, but not in the kidney. However, hepatic Mn concentration hardly increased, whereas renal Mn concentration increased dose-dependently. HPLC/ICP-MS analysis on the distribution of metals in hepatic cytosol of Mn-treated mice revealed that the major metal bound to MT-I and MT-II was not Mn, but Zn. A time-course experiment showed that hepatic Mn concentration reached the peak at 1 h after the injection and then declined quickly. Following the transient accumulation of Mn in the liver, serum interleukin-6 (IL-6) concentration reached the peak at 6 h after the injection, and then serum amyloid A, the indicator of hepatic inflammation, gradually increased. However, serum IL-1β, TNF-α, GPT or BUN did not increase after the $MnCl_2$ injection. To confirm the involvement of IL-6 in MT induction by $MnCl_2$, IL-6 null and control mice were injected with $MnCl_2$. In IL-6 null mice, no increase in hepatic MT or serum amyloid A was observed. These results suggest that the induction of hepatic MT in mice by $MnCl_2$ administration is entirely dependent on the production of inflammatory cytokine, IL-6.

149 EFFECT OF MANGANESE ACETATE ON THE SPERM PROFILE AND MATING PERFORMANCE IN SD RATS.

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Manganese plays a pivitol role as a ubiquitous constituent in the environment that is beneficial or essential at low intake levels while inhalation or oral exposure to high levels can cause adverse effects. In the present study, the effect of manganese exposure on the reproductive and testicular function in Sprague-Dawley rats was investigated. Oral administration of manganese acetate was given daily to male and female Sragues-Dawley rats at 0(control/control positive), 76, 156 and 306 mgMn/kg body weight for a period of 63 days prior to mating, 5 days during mating and 17 days of pregnancy until necropsy. At necropsy, the end points of target organ toxicity examined for the treated males included; organ weights, percent sperm motility, sperm concentration in testis and caudal tissue. Crossover mating trials were accomplished between high dose animals of each sex and control rats of opposite sex. Results of the crossover were compared to mating within control positive groups to determine which sex and dose group were affected. End points for females included; organ weights, fertility assessments, total implants, and resorptions. Our data results demonstrated that there was a decrease in organ weight in both sexes. There was a significant reduction in testicular and caudal sperm counts in all groups. Sperm motility was reduced in all groups, but was significant in rats dosed at 306 mgMn/kg. Fetal observations showed lower values in pregnancy rates, uterine weights, and the number and weight of fetuses. Control positive females mated with control positive males had a pregnancy rate of 78.5% and the average resorptions of 0.33/litter. The pregnancy rate was 60% and an average resorptions were 1.33/litter in control females mated with treated males. Treated females mated with control males had a pregnancy rate of 75% and resorption of 1.08/litter. This study demonstrates manganese to affect the reproductive and fertility profile of both male and female rats, but comparatively, male rats are affected more than females. Supported by ATSDR U50/ATU398948-10

150 HEXAVALENT CHROMIUM TOXICITY IN HUMAN LUNG CELLS, CHINESE HAMSTER OVARY CELLS AND A549 CELLS: CURRENT CELL CULTURE MODELS ARE INADEQUATE.

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Hexavalent Chromium (Cr (VI)) is a well-established human carcinogen and exposure to it is widespread. Cr (VI) induces genotoxicity and cell cycle arrest, but the specific mechanisms involved are uncertain. Most Cr (VI) studies have focused on

Chinese hamster ovary cells (CHO) or human tumor lines such as A549 cells. But these are known to have altered cell cycle machinery and may not be suitable models for such mechanistic investigations. We evaluated the cytotoxicity of Cr(VI) in these lines and in primary human bronchial fibroblasts (PHBF). We found that Na_2CrO_4 (NC) was cytotoxic to PHBF, inducing 79, 59, 18, and 0 percent survival at 1, 2.5, 5, and 10 uM, respectively. By contrast NC was less toxic to A549 cells inducing 69, 68, and 22.3 percent survival at 2.5, 5, and 10 uM, respectively. CHO cells were much more similar to PHBF with NC inducing 79, 69, 35, and 0 percent survival at 1, 2.5, 5, and 10 uM, respectively. We also found NC induced cell cycle arrest (expressed as mitotic index) in PHBF. Specifically, in PHBF, the mitotic index was 44, 46, 26 and 2% at 1, 2.5, 5 and 10 uM, respectively. There was much less arrest in A549 cells, as the mitotic index did not change significantly with dose (58, 59, 52 and 43% at 0.5, 2.5, 5 and 10, respectively). CHO cells were less sensitive than PHBF to NC-induced arrest at low doses (78% and 66% at 0.5 mM and 1 mM, respectively), but were similar at the two highest doses (19% and 4% at 5 and 10 uM, respectively). In conclusion, CHO cells and A549 cells appear to be inadequate models for investigating the mechanisms of cytotoxicity and cell cycle effects of Cr (VI) in human bronchial cells. New cell culture models are clearly needed to allow for mechanistic investigations of the cell cycle effects of Cr (VI) and other metals. Future efforts in our laboratory are aimed at assessing the utility of CHO and A549 in evaluating Cr (VI) genotoxicity and developing new human bronchial cell culture models.

151 A NEW HUMAN BRONCHIAL CELL CULTURE MODEL FOR INVESTIGATING METAL-INDUCED LUNG CARCINOGENESIS.

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Metals are an important public health concern. Several metals are lung carcinogens and pose a significant risk for both occupational and environmental exposure. Five metals are listed in the "Top 20 Hazardous Substances" by the Environmental Protection Agency (EPA) and the Agency for Toxic Substances and Disease Registry (ATSDR), and five metals are listed as "carcinogenic to humans" by the International Agency of Cancer Research (IACR). However, the mechanisms underlying metal-induced lung cancer are poorly understood. This is largely due to the lack of adequate human bronchial cell culture models. Accordingly, we developed a new model system of human bronchial cells by introducing hTERT, the catalytic subunit of telomerase, into primary human bronchial fibroblasts (PHBF). We have isolated stable, monoclonal cell lines that demonstrate reconstitution of telomerase activity (shown by expression of ectopic hTERT using a telomeric repeat amplification protocol (TRAP)). These cell lines have been characterized as having: an extended *in vitro* life-span, a normal karyotype, anchorage dependent growth, contact-inhibited growth, and a need for exogenously supplied growth factors. Moreover, we find that both particulate and soluble hexavalent chromium induce similar amounts of cytotoxicity, clastogenicity and cell cycle delay in these lines and the parent PHBF cells. We are currently using this model to study the toxicology of hexavalent chromium, cadmium, and arsenic. Since telomerase does not compromise the function of p53 or pRB, we feel this is an excellent system for studying the cell cycle and DNA damage response of these as well as other carcinogens implicated in the development of lung cancer.

152 CYTOTOXICITY AND GENOTOXICITY OF CR(VI) IN HUMAN BRONCHIAL CELLS.

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Hexavalent chromium (Cr(VI)) is a particular class of compound that is now common in the environment as a consequence of industrial pollution. Cr(VI) is widely accepted as a human bronchial carcinogen, but its carcinogenic mechanisms are poorly understood. One reason for this is the lack of information of the effects of Cr(VI) in human bronchial cells. There is only very sparse data concerning Cr(VI) and human bronchial cells. Accordingly, we investigated the cytotoxicity and genotoxicity of Cr(VI) in cultured primary human bronchial fibroblasts (PHBF). Cytotoxicity was assessed using a colony-forming assay. Using sodium chromate (SC) as a model Cr(VI) compound we found that SC induced dose dependent cytotoxicity in PHBF with 1, 2.5, 5, and 10 uM inducing 79, 59, 18 and 0 percent relative survival respectively. We also found that SC induced genotoxicity in PHBF in a dose dependent manner using the comet assay. Specifically, 0.5, 1.0, 2.5, 5.0, 10.0, and 25 uM induced a 1.4, 1.5, 2.0, 2.1, 2.3, and 2.8 fold increase in the extent of the comet relative to control. Thus we can conclude that Cr(VI) is cytotoxic and genotoxic to PHBF in a dose dependent manner. Future investigations are aimed at investigating the genotoxicity of particulate Cr(VI) compounds and understanding the time-course of damage formation and repair.

153 CHROMIUM(III) AND CHROMIUM(VI) STIMULATE cAMP-MEDIATED CELL SIGNALING IN CELL CULTURE AND *IN VIVO*.

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Cr(III) is an essential trace element at normal dietary levels whereas Cr(VI) can be a respiratory carcinogen following chronic, high dose, occupational inhalation exposure. We had previously observed selective Cr(VI) effects on expression of the glucocorticoid receptor (GR) and cAMP-regulated PEPCK gene as well as artificial gene constructs containing GR regulatory elements (GREs) in rat hepatoma H4IIE cells. Some of these effects could be mimicked by treatment of cells with Cr(III), which does not enter cells, or by cAMP itself. Both Cr(III) and Cr(VI) also stimulated strictly cAMP response element (CRE)-driven gene expression. We have investigated the basis for these effects further both in H4IIE cells in culture and in 14 day chick embryo liver *in vivo*. In cell culture, Cr(III) was not taken up by cells, and the effects of Cr(VI) on cAMP could be completely accounted for by extracellular reduction of Cr(VI) to Cr(III). However, Cr(VI) also had effects on expression of PEPCK and other inducible genes that are independent of cAMP pathways, presumably as a result of uptake followed by intracellular reduction of Cr(VI) to Cr(III) and/or reactive intermediates. The Cr(III) stimulation of cAMP was blocked by Rp-cAMPs, a specific inhibitor of adenylyl cyclase (AC), and could be mimicked by forskolin which stimulates AC. In the chick embryo, low doses (1-50 µmol/kg) of chromium chloride or chromium picolinate stimulated cAMP in liver within 30 min, similar to effects of forskolin. Cr(VI) treatment also stimulated cAMP in liver, although at higher doses, consistent with the predicted extracellular reduction to Cr(III). Previously, Cr(III) was thought to act as an essential trace element principally by enhancing insulin receptor signaling. These results suggest that Cr(III) stimulation of cAMP signaling may also be an important physiological effect of Cr(III) — and Cr(VI) reduced extracellularly to Cr(III) — leading to alterations in cell signaling and gene expression. (NIEHS (SBRP) ES07373)

154 EFFECTS OF CHROMIUM PICOLINATE ON ANTIOXIDANT ENZYME LEVELS IN RATS.

M. Mahboob, L. McNeil, T. Tolliver and L. Ogden. *Biomedical Sciences, Tuskegee University, Tuskegee, AL.* Sponsor: R. Dalvi.

The purpose of this study was to determine the effects of chromium picolinate on body weight, feed efficiency, lipid peroxidation (LPO) and antioxidant enzymes levels, i.e. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH) and glutathione reductase (GR) in the liver and kidneys of the rat. Male rats weighing 250-300 g were divided into 4 groups of three animals each and exposed orally to chromium picolinate in the doses, 0.00 (control), 2.66 (low), 5.32 (mid) and 10.64 (high) µg/kg/day respectively for 21 days. Both body weight and feed efficiency were reduced, kidney-body weight ratio increased, but, the liver-body weight ratio was unaffected in the treated groups. The liver SOD, GPx, GSH were decreased in all the treated groups and the CAT level also decreased, but only in the high dose group. The kidney SOD and CAT levels decreased in all the treated groups, similarly, GSH and GR levels were reduced in the mid and high dose treated groups. Results revealed that there was a trend towards increase in the LPO level in both the tissues in all the treated rats. This data indicated that the low level of chromium picolinate reduces the body weight and also affects antioxidant enzyme systems of the liver and kidneys in the rat.

155 MECHANISMS FOR SELECTIVE ACTIVATION OF JNK BY LOW LEVELS OF CHROMIUM(VI).

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Inhaled hexavalent chromium Cr(VI) can promote fibroproliferative disease, airway hypersensitivity, and possibly lung cancer through poorly defined mechanisms. Therefore, cultured epithelial cells were used to examine the hypothesis that nontoxic Cr(VI) exposures activate gene transcription through specific oxidant-sensitive signaling pathways. These studies demonstrated that 1-10 µM Cr(VI) increased reactive oxygen species and selectively activated JNK, relative to ERK or p38 MAP kinase. Stimulation of JNK by these levels of Cr(VI) was comparable to the effects of 1.0 mM H_2O_2. However, in contrast to H_2O_2, 10 µM Cr(VI) was only marginally toxic. JNK activation was blocked by pre-incubating the cells with either N-acetylcysteine or PP2, a selective inhibitor of the Src family of tyrosine kinases (SFKs). Treatment with rotenone to block mitochondrial respiration or with exogenous catalase only partially attenuated Cr(VI)-induced JNK activity. Neither Cr(VI) nor H_2O_2 activated JNK in SFK deficient cells. H_2O_2, but not Cr(VI) activated JNK in cells containing only Src. Transient transfection with wild type or mutant p130cas indicated that both Cr(VI) and H_2O_2 require this adapter protein

and Src substrate to activate JNK. These data suggest that Cr(VI) signals through cellular oxidation to selectively activate SFKs and downstream JNK signaling. In contrast to previous studies, these findings in pulmonary cells demonstrate mechanisms for the exclusive sensitivity of the JNK pathway to the effects of Cr(VI). *Supported by NIEHS grant ES07373 and ES10638.*

156 ADAPTIVE ANTIOXIDANT RESPONSES IN HUMAN A549 LUNG CELLS TREATED WITH HEXAVALENT CHROMIUM.

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Hexavalent chromium (CrVI) is genotoxic and has been classified by the International Agency for Research on Cancer as a Group 1 carcinogen. CrVI is reduced intracellularly to CrIII involving the production of various intermediate species, including CrV and CrIV and accompanying the production of reactive oxygen species (ROS). The resulting oxidative stress is believed to be responsible for many of the cellular effects caused by CrVI, and may contribute to changes in gene expression. In the current study, CrVI-mediated changes in the expression of several stress-related genes were investigated in human A549 lung cells. In association with oxidative DNA damage, sodium dichromate gave a dose-dependent increase (3.4-fold increase; 100μM) in antioxidant capacity of cells at 2h as measured by the inhibition of pholasin® oxidation and bioluminescence (ABEL assay; Knight Scientific Ltd.,). There was no decline in intracellular ATP concentrations. A persistent increase in heme oxygenase (HO-1) (within 4h) and a transient elevation of cytosolic thioredoxin (TRX) (1.8-fold increase at 2h as measured by densitometry) protein levels was also seen by western blotting. Nuclease protection assays confirmed the elevation of HO-1 mRNA but also demonstrated that the mRNA for various other antioxidant systems (glutathione reductase, Mn-superoxide dismutase and NAD(P)H quinone oxidoreductase) along with the house-keeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was not elevated. In conclusion, in association with DNA damage, treatment with sodium dichromate appears to elevate selective antioxidant defences that may contribute to protective adaptation to exposure. (Sponsored by the Colt Foundation).

157 EFFECTS OF ZINC CHLORIDE ON REPRODUCTIVE PARAMETERS OF CD-1 MICE.

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The effects of low doses of zinc chloride ($ZnCl_2$) on the reproductive performance have not been well described. The major objective was to determine the effects of low doses of $ZnCl_2$ on reproductive performance in a mammalian species. Life stages evaluated were puberty, cohabitation, gestation, and lactation. Both males and females were exposed to either 0.00, 0.78, 1.56, or 3.125 mg/kg $ZnCl_2$ daily by oral gavage until they were terminated. Males were sacrificed after cohabitation and females after lactation. Reproductive parameters evaluated were mean litter size, pup viability index, pup litter weight, live births, clinical signs, fertility index, post partum dam body weights, food consumption and male and female body weights. Abnormal nursing and nesting behaviors were observed in the mid and high dose groups. This was characterized by hyperactivity, absence of nest building, failure to allow pups to nurse and killing pups. There were no significant differences in the F_0 male and female body weight gain or food consumption. There was a dose dependent trend toward decreased implantation efficiency. Litter size was greatest in the control group and decreased as dose increased. There was a trend toward a decrease in the number of live births in the mid and high dose groups. Also, there was a trend toward decreased numbers of live births in the mid and high dose groups. Mortalities occurred in all treatment groups but were greatest in the low group, high group and the mid dose groups, respectively. These data suggest that zinc adversely affects some reproductive parameters when mice were exposed to oral doses as low as 0.78 mg/kg. (Supported by MHPF/ATSDR Cooperative Agreement # U50/ATU3922660009).

158 THE OVEREXPRESSION OF MT-3 IN THE NORMAL HUMAN BLADDER CELL LINE, UROTSA.

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The third isoform of metallothionein (MT-3) has a limited tissue distribution, being confined primarily to neural tissue and the kidney; however, overexpression of this protein is found in many cancers of the breast, prostate and bladder. Overexpression of MT-3 has been demonstrated in bladder tumors with the levels correlating to the type and grade of the tumor, whereas MT-3 expression in the normal human bladder was undetectable. Recently a new normal bladder cell line, urotsa, was characterized as a useful *in vitro* model system of human urothelium and found not to express MT-3. The consequence of MT-3 overexpression in urotsa cells was tested by stable transfection with MT-3 under control of the CMV promoter. Twenty clones were isolated and analyzed for the expression of MT-3. RT-PCR confirmed the expression with products being identified at 25 cycles of PCR. Similar amounts of PCR products were obtained using primers for glyceraldehyde 3-phosphate dehydrogenase indicating that the level of MT-3 expression was similar to that of a common house-keeping gene. The levels of MT-3 protein were surprisingly low, however, with levels often pushing the limit of detection (0.5 ng/μg protein). Since MT-3 has been successfully overexpressed in many cell lines of the prostate, kidney, and breast, the nearly non-existent levels of MT-3 in the urotsa clones suggested that there was the potential for selection against MT-3 expression. MT-3 was then overexpressed using the regulated vector system, LacSwitch, in which stable tansfectants can be selected under MT-3 dormant conditions and then induced with the exogenous agent isopropyl β-D-thiogalactopyranoside (IPTG). Using this vector system, levels of MT-3 protein were significantly higher (average 4.13 ng/mg protein).

159 METALLOTHIONEIN ISOFORM 3 (MT-3)INHIBITS GROWTH AND INCREASES CHEMOTHERAPEUTIC RESISTANCE OF BREAST CANCER CELLS.

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MT-3 is unique among the members of the metallothionein gene family in that its expression in normal cells is limited and it has a neuronal growth inhibitory activity. The present study was designed to determine if overexpression of MT-3 and 1E influences the growth and chemotherapeutic drug resistance of breast cancer cells. The cell lines MCF-7, T-47D, Hs 578T and MCF-10A were stably transfected to overexpress the MT-3 or MT-1E genes using the pcDNA3.1/Hygro (+) vector. Overexpression of the genes was confirmed in 5 independent clones by a combination of RT-PCR and immuno-blot analysis. Growth and chemotherapeutic drug resistance were determined by cell counts and colony formation assays. Overexpression of MT-3 was demonstrated to inhibit the growth rate of the breast cancer cell lines by approximately 50% compared to vector-only controls ($\alpha<0.001$ for each comparison) but to have no effect on the growth rate of non tumorigenic MCF-10A breast cells. For all cell lines, the overexpression of MT-3 resulted in increased resistance to: Cd^{+2}, cisplatin, doxorubicin, paclitaxel and methotrexate. Overexpression of the 1E isoform conferred a resistance to the above chemotherapeutic agents, but did not cause an inhibition of cell growth.

160 STIMULATION OF CYTOKINE PRODUCTION IN MACROPHAGE BY ZINC *VIA* METALLOTHIONEIN.

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We previously reported that human cytomegalovirus major immediate-early promoter activity is controlled by intracellular zinc levels *via* metallothionein (MT)-mediated regulation of NF-kappa B activity (Antiviral Res., 47, 207-214, 2000; Toxicol. Appl. Pharmacology, 173, 146-153, 2001). This transcriptional factor is an important protein involved in expression of several cytokine genes and regulation of various cell functions. The expression of MT is easily changed in many kinds of toxicological responses. In the present study, we evaluated the effects of MT on the expression of proinflammatory cytokines in macrophages. We used peritoneal exudate macrophages (PEMs) obtained from MT+/+ or MT-/- mice. Secretion of tumor necrosis factor (TNF) stimulated by lipopolysaccharide (LPS) from MT-/- PEMs was significantly less than wild-type control. LPS-stimulated expression of TNF-alpha was decreased in MT-/- PEMs. In electrophoretic mobility shift assays, LPS-stimulated activation of the DNA-binding activity of NF-kappa B is also decreased in MT-/- PEMs. *in vivo* response was also evaluated in cell-free ascites fluid harvested 1 h after ip injection of LPS. The response of PEMs in MT-/- mice was less than in wild-type mice *in vivo* in the same manner as *in vitro*. These findings suggest that the LPS-induced production of TNF in mouse peritoneal macrophages is upregulated by MT through the modulation of NF-kappa B activity. Expression of other inflammatory cytokines, such as interleukin (IL)-1alpha and IL-6, is also downregulated in MT-/- PEMs. The function of PEMs was changed by MT deficiency in terms of LPS-induced cytokine responses. Thus, MT plays an important role in the LPS-induced activation of PEMs. Since MT is induced by various xenobiotics, it is possible that some chemicals modulate host defense system through the induction of MT.

161 THE ROLE OF METALLOTHIONEIN IN APOPTOSIS INDUCED BY ZINC IONOPHORE.

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Various cellular functions are influenced by essential trace elements such as the divalent cation zinc. It has been shown that excessively released zinc induces neuronal cell death associated with apoptosis. The metal binding protein, metallothionein (MT), is probably involved in zinc homeostasis since it typically binds large amount of zinc. Recent work has provided new role of MT in regulation of cell cycle and apoptosis. In this study, we investigated that excessive zinc accumulation in fibroblast cells induces apoptosis and necrosis depending on zinc concentration, and MT inhibits the apoptosis induced by zinc. Intercellular zinc concentrations in MT-I and -II null (MT-/-) and wild-type (MT+/+) fibroblast cells exposed with $ZnCl_2$ for 2hours were not different from non-treatment cells. The data indicate that the physiological concentration of zinc in cells is strictly controlled. Then we used Na pyrithione, zinc ionophore, and it increased the zinc concentration at 2.5 fold compared to the control, and co-existence of $ZnCl_2$ and Na pyrithione in the cell culture medium further increased the zinc concentration (7.5 fold) in both cells. MT-/- cells were more susceptible to Na pyrithione than MT+/+ cells as assessed by MTT. But sensitivity of co-exposure with $ZnCl_2$ and Na pyrithione was not different between MT+/+ and MT-/- cells. DNA fragmentation, one of the hallmarks of apoptosis, was induced by Na pyrithione, although co-treatment of $ZnCl_2$ and Na pyrithione did not induce DNA fragmentation. The DNA fragmentation by Na pyrithione in MT-/- cells was stronger than that in MT+/+ cells. These results indicate that the concentration of zinc in cells determines mode of zinc-induced cell death. MT inhibits the apoptosis induced by zinc.

162 EFFECT OF ZINC CHLORIDE ON THE HEMATOLOGICAL AND CLINICAL CHEMISTRY PARAMETERS DURING A BREEDING TRIAL OF CD-1 MICE.

T. Graham, M. Mahboob, A. Atkinson, L. Ogden, M. Hammersley, N. Sarker, A. Casimire and F. Johnson. *Biomedical Sciences, Tuskegee University, Tuskegee, AL.* Sponsor: R. Dalvi.

The effects of low doses of zinc chloride ($ZnCl_2$) on the reproductive performance have not been well described. The major objective of this study was to determine the effects of low doses of $ZnCl_2$ on the reproductive performance in a mammalian species. A breeding trial was conducted where males and females were divided into four groups and exposed to 0.00, 0.78, 1.56, or 3.125 mg/kg of $ZnCl_2$ by oral gavage. The major objective was to determine the effects of low doses of $ZnCl_2$ on clinical pathology parameters that may adversely influence reproductive performance. The hematological parameters included WBC, RBC, HGB, HCT, MCV, MCH and MCHC. The clinical chemistries included, Na, K, Cl, PO_4, Ca, AST, ALT, Albumen, Globulin, Total Protein, Amylase, Creatinine, CK, BUN, and Glucose. Blood samples were collected at scheduled sacrifices (post cohabitation for males and post lactation for females). The most relevant changes in hematological parameters were seen in males. High dose males had decreased MCH and MCV. Although not significant, there were trends toward increased WBC counts in all $ZnCl_2$ treatment groups. Few significant changes were seen in clinical chemistries in either sex. However, creatinine was elevated in high dose males and CK was elevated in high and mid dose males. In summary, oral exposure to $ZnCl_2$ at the levels in this experiment did not alter hematological or clinical chemistry parameters to the degree necessary to influence reproductive performance. (Supported by MHPF/ATSDR Cooperative Agreement # U50/ATU3922660009).

163 THE EFFECTS OF ZINC CHLORIDE ON THE ANTIOXIDANT ENZYME ACTIVITY IN F_1 MALE MICE.

M. Hammersley, L. Ogden, M. Mahboob, A. T. Khan and T. Graham. *Biomedical Sciences, Tuskegee University, Tuskegee, AL.* Sponsor: R. Dalvi.

Trace amounts of zinc are required for functional activity of several biological molecules; however, excessive amounts may be toxic to mammals. This study was conducted to determine if low levels of zinc chloride ($ZnCl_2$) given to F_0 generation affects antioxidant enzyme activity in the brain, testis, liver and kidney of the F_1 offspring. CD-1 males (70 days) and females (99 days) were exposed to 0.000 (control), 0.78 (low), 1.560 (mid), and 3.125 (high) mg/kg/day of $ZnCl_2$ *via* oral gavage. After weaning, F_1 males were weighed and sacrificed. Brain, testes, liver and kidneys were removed and assayed for the catalase (CAT), gluthathione peroxidase (GPx) and superoxide dismutase (SOD) activities. In the brain the SOD activity increased, while the GPx and CAT activities decreased in all treatment groups. Liver SOD and GPx activities increased in all treated groups, except at the low dose, in which the GPx activity decreased. CAT activity decreased in low and high dose and increased at the mid dose. The kidney SOD activity increased in all treated groups. GPx activity in the kidney increased in low and mid dose groups but decreased in high dose groups. CAT increased in all groups but decreased in low dose group. However, these values were not significant. In the testes, there was a dose dependent increase in SOD activity. The GPx increased at the low dose whereas the CAT activity decreased in all treatment groups. In conclusion this study indicates that low doses of zinc chloride alters antioxidant enzyme activity in F_1 male mice when parents were exposed to zinc.

164 IRON AUTOXIDATION AND FREE RADICAL GENERATION: EFFECTS OF BUFFERS, LIGANDS, AND CHELATORS.

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If not tightly controlled iron can cause various toxicities as a result of its ability to mediate the deleterious oxidation of biomolecules, e.g., DNA, lipid, protein, and small molecules, such as ascorbic acid and biogenic amines. The iron-mediated oxidation of biomolecules can be accentuated by some chelators. The liganding environment of iron, including the pH of the solution and chelation, regulate its reactivity. In this study we confirmed that, in general, the rate of Fe(II) autoxidation increases as the pH of the solution is increased. However, we demonstrated that chelators which provide oxygen ligands for the iron can override the affect of pH. Additionally, the stoichiometry of the Fe(II) autoxidation reaction varied from 2:1 to 4:1, dependant upon the chelator. No partially reduced oxygen species were detected during the autoxidation of Fe(II) by ESR using DMPO as the spin trap. However, upon the addition of ethanol to the assay, the DMPO:hydroxyethyl radical adduct was detected. The oxidant formed during the autoxidation of EDTA:Fe(II) was shown to have different reactivity than the hydroxyl radical, suggesting that some type of hypervalent iron complex was formed. In addition, ferrous iron was shown to directly reduce some quinones without the reduction of oxygen.

165 DOSIMETRY OF BERYLLIUM IN AN ANIMAL MODEL BY ACCELERATOR MASS SPECTROMETRY.

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A method using accelerator mass spectrometry (AMS) was developed to measure low levels (femtomoles) of beryllium in biological samples. This method provides the sensitivity to investigate macromolecular complexes formed with beryllium at low exposure doses and will provide further understanding about the molecular targets involved in beryllium disease. Berylliosis is a debilitating, progressive and potentially fatal lung disease that develops in individuals exposed to beryllium. Proof of the method was tested by administering 0.05, 0.5 and 5.0 µg 9Be and 10Be by IP injection to 30 g male ICR mice. The mice were euthanized after 24 h and blood, femurs, feces, urine, kidneys, spleen, liver, thymus and lung were prepared for AMS analysis by acid digestion. Highest levels of Be were found in the liver and the spleen (6.0 and 2.0% of whole mouse dose, respectively) while the lowest levels were found in blood, lung and thymus. Beryllium levels were dose-dependent in the spleen and liver. The detection limit of Be in tissue by this method is approximately 2 amol and the analysis was linear over 2 orders of magnitude. Possible sample size effects for measuring Be by AMS showed that similar results were obtained when using samples that were between 3.0 and 150 mg of dosed liver tissue. Precision of 8 replicates of pooled liver tissue was 5% while the variability between 8 dosed livers was 10%. These results show that routine quantification of atto- to femtomole levels of Be in tissues is possible. This method should enable future studies to understand the molecular dosimetry and mechanisms of Be toxicity in biological studies. This work performed under the auspices of the USDOE by LLNL (W- 7405-ENG-48) with support from the USDOE/OBER.

166 LIPID PEROXIDATIVE ACTIVITY OF TWO INHALED VANADIUM(V) CONCENTRATIONS (0.01M AND 0.02M) IN LUNG, TESTES, LIVER AND KIDNEY.

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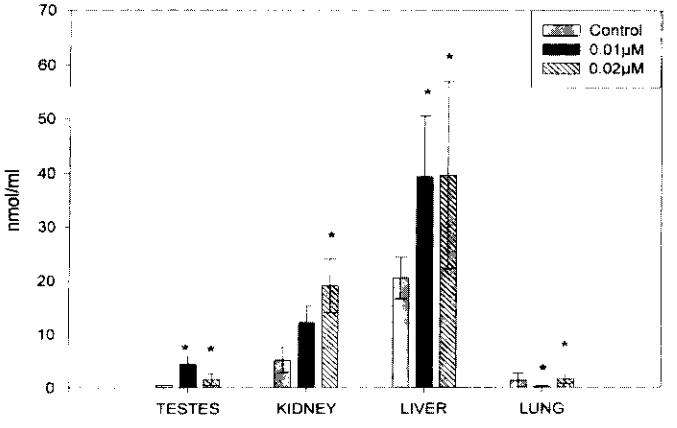
Metals have become a problem in some countries(1). A less studied metal is vanadium -a highly oxidant metal- which could be an essential or a toxic element. One of the vanadium sources in the air is the combustion of fossil fuel products.

Mexican Maya oil has one of the highest V concentrations. Mexico City with three million circulating vehicles, has an increase in V over a thirty years period in measured in human lungs. This information made us to investigate the induction of lipid peroxidation in four organs (lung, testes, liver and kidney) in a mice model after the inhalation of V2O5. Methodology: CD-1 mice inhaled (1hr) 0.01M or 0.02M during 4 weeks twice a week. Controls inhaled only the vehicle. Mice were sacrificed after the first inhalation, and each week. Peroxides were quantified by light spectrometry using a K-assay LPO-CC. Results: Lung and testes control mice reported the lowest basal concentrations. An statistically significant increase was observed in testes at 0.01M. Kidney and liver showed an increase in lipid peroxides in a dose-dependent manner. Liver at 0.02M reported the highest lipid peroxidation concentrations. Conclusions: The inhalation of V induced lipid peroxidation in the liver and the kidney, with no differences related with the dose. Lung and testes reported almost no peroxidative activity as a consequence of V inhalation. These differences might be related with the organ content of antioxidant agents, such as glutathione, the ability to concentrate the metal, or other specific physiological characteristics of the organs which requires further anlalysis.

LIPID PEROXIDATION IN TESTES, KIDNEY,LIVER AND LUNG TISSUES AFTER VANADIUM INHALATION



* Mann Whitney U Test p<0.001vs. controls

167 GENOTOXICITY STUDIES OF TWO HISTORICAL SAMPLES OF NICKEL REFINERY DUST.

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The INCO nickel refinery in Clydach, Whales, U.K., was actively in operation from 1901 until the 1940's. Since 1901, there have been a total of 365 cases of cancers reported in the workers of this refinery, including 85 nasal cancers and 280 lung cancers. From 1901 to 1923, the incidence of these cancers was very high. The INCO refinery then changed the process of nickel refining after 1923 (Draper, 1994, ETP). The change in the refining process eliminated the orcelite component. The incidences of cancers were subsequently reported to be greatly reduced from 1925-1930. A refinery dust sample was taken in 1920 and another in 1929. Both samples contain primarily NiO. The main difference between the two dust samples is the presence of a nickel arsenide (Orcelite) in the 1920 sample. The arsenic content in the 1920 sample is ~10% and in the 1929 sample is only ~1%. We hypothesized that it is the nickel arsenide component of the 1920 sample that was responsible for the nasal and respiratory cancers in the refinery workers. A pure sample of the nickel arsenide (orcelite) was obtained and used for comparison. Our results show that all of the samples were taken up by the cells by phagocytosis at similar rates. both the 1920 and the 1929 samples, and the pure orcelite sample are cytotoxic to 10T½ mouse embryo cells. The LC_{50} values are (3.2 ± 0.7)µg/ml for the 1920 sample, (1.9 ± 0.2)µg/ml for the 1929 sample, and (2.5 ± 0.0)µg/ml for the pure sample of orcelite. In support of our hypothesis, we found that the 1920 sample is able to induce morphological transformation in a dose-dependent manner, with 9 foci per 20 dishes treated with the highest concentration of 7.5µg/ml. We found that the 1929 sample did not induce morphological transformation. Therefore, the data provides evidence that the 1920 sample has carcinogenic potential, consistent with induction of nasal and respiratory cancers in the refinery workers before 1923. Further investigation is in progress with the pure sample of orcelite to determine its transforming ability.

168 UTILIZING ARRAYS TO CHARACTERIZE GENERALIZED AND SPECIFIC STRESS REPONSES IN MUMMICHOGS.

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We are using mummichogs *(Fundulus heteroclitus)* as a model organism to study the effects of generalized stress and specific contaminant stress. These estuarine minnows are a good indicator species because they have a limited home range and complete their entire life cycle in the estuary. We have been examining two estuarine sites near Charleston, South Carolina that are contaminated with a suite of toxicants. One of the sites is impacted with metals, with trivalent and hexavalent chromium being the dominant contaminants, while the other site has been contaminated with PAHs including pyrene, fluoranthene, and anthracene. To ascertain the effects of these contaminants, we exposed mummichogs to these five chemicals individually, and examined altered gene expression in their livers by differential display. We used the differentially-expressed fragments, as well as other known stress-responsive genes, to develop cDNA arrays. These additional fragments included genes involved in detoxification such as CYP1A and catalase, genes involved in the immune response including cyclooxygenase-II, iNOS, and the major vault protein, as well as other known mummichog sequences such as the CFTR gene and a sodium/potassium ATPase. Upon screening the arrays with control and trivalent chromium-exposed fish, we have found inductions in CYP2N2 and heart-fatty acid -binding protein. We hope to use this information and the cDNA arrays to develop markers of generalized toxicant stress as well as markers that are specific for a particular contaminant or class of contaminants.

169 SELECTION OF FILTER MATERIAL FOR METAL COMPOSITION ANALYSES OF AMBIENT PARTICULATE MATTER SAMPLES BY ED-XRF.

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Energy-dispersive X-ray Fluorescence (ED-XRF) is becoming a popular chemical analysis technique in large-scale air sampling campaigns. Filters or substrates used to collect ambient particulate matter (PM) for XRF analysis should have the following characteristics: (1) low mass to reduce scattering of incident radiation thus minimizing background count, (2) low concentrations of elements to be measured, and (3) low penetration of particles below the surface. Five types of commercially available filter material were analyzed for background metal content using a JVAR EX-6600 AF instrument. These filters were quartz fiber, Whatman paper, Teflon on polyolefinic ring support, Teflon on fiber support, and polycarbonate membrane. A sample of results is shown in Table 1. Quartz fiber filters were unacceptable for PM metal analysis due to high background metal concentrations and high penetration factor. Additionally, light elements such as sodium, magnesium, and aluminum could not be analyzed due to high silicon concentration. Whatman paper also had high metal concentrations in blanks although much lower than quartz fiber. Filter materials that have been found most suitable were the thin membrane type: Teflon and polycarbonate, as they have lowest background and penetration. Caution should be excercized while sampling on Teflon with fiber support as sampling on the "wrong", i.e. fiber side of the filter, is a common mistake. Supported in part by EPA R827351. TABLE 1. Selected background metal concentrations in filter material (ng/cm ^{2}).

	S	V	Fe	Ni	Cu	Zn
Quartz fiber	1074	48	1060	54	134	126
Whatman paper	200	0.77	100	4.4	85	1.3
Teflon on polyolefinic ring	15.7	1.8	19.7	4.8	27	0.60
Teflon on fiber support	16.6	3.3	19.2	2.3	25	72
Polycarbonate	15.7	1.8	9.3	2.7	7.5	1.7

170 THE EFFECTS OF AN ORGANOPHOSPHATE(OP)-CARBAMATE(CB) PESTICIDE MIXTURE ON CORE TEMPERATURE AND MOTOR ACTIVITY IN THE RAT.

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Pesticide risk assessment has traditionally been based on the toxicological response to single agents. Dose-additivity has been the default in risk assessment evaluations of pesticides with a common mechanism of action, but there could be supra-additive or infra-additive interactions of pesticide mixtures. We have found that rats exposed to anticholinesterase OP or CB pesticides display a transient hypothermia

followed by a delayed fever. Thus, the hypothesis of additivity of an OP/CAR mixture may be assessed using thermoregulatory endpoints. A fixed-ratio ray design was developed from single chemical dose-responses to assess the effects of a mixture of the OP chlorpyrifos (CHP) and the CB carbaryl (CAR). Adult, male Long-Evans rats were surgically implanted with radio transmitters (Data Sciences) to monitor core temperature (Tc) and motor activity (MA). After recording a 24 hr baseline of Tc and MA, rats were dosed orally with corn oil, 30 mg/kg CHP, 75mg/kg CAR, or one of the following 2:1 mixtures of CHP:CAR; 4.7:2.3, 9.3:4.7, 14.0:7.0, 18.7:9.3, or 23.3:11.7 mg/kg. Exposure to pure CHP and CAR yielded an acute, marked reduction in Tc followed by a delayed fever for at least one day post dose. The hypothermic response to the binary mixtures was diminished compared to the pure chemicals. A mixture of 23.3:11.7 mg/kg led to a hypothermic response half that of 30 mg/kg CHP (assuming dose-additivity, the hypothermic response of these two treatments should be equal). However, the CHP:CAR mixtures >14.0:7.0 mg/kg led to a delayed fever similar to that elicited by pure CHP. MA post dosing was reduced in the 30 mg/kg CHP group, whereas the mixtures did not affect MA. Overall, there is an infra-additive response to a mixture of CHP:CAR when assessed by the hypothermic response. This response is mediated through stimulation of cholinergic thermoregulatory pathways. The delayed fever, mediated by non-cholinergic systems, is unaffected by the CHP:CAR mixture. This abstract does not necessarily reflect USEPA policy.

171 *IN VITRO* INHIBITION OF RAT BRAIN AND SERUM CHOLINESTERASE BY BINARY MIXTURES OF SIX ORGANOPHOSPHATES.

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Rat brain and serum were tested for cholinesterase (ChE) inhibition from binary mixtures of oxons of 6 phosphorothionate insecticides: methyl parathion (methyl paraoxon), parathion (paraoxon), azinphosmethyl (azinphosmethyl-oxon), chlorpyrifos (chlorpyrifos-oxon), malathion (malaoxon) and diazinon (diazoxon). Oxons were tested at concentrations yielding 10, 20 or 30% inhibition by the individual compounds. Serum was tested in the presence of EDTA so that A-esterases were not functional. A dose-additive model was used to predict the expected percent inhibition of the mixture; this model expressed the concentration of one oxon in the pair in terms of the concentration equivalents of the other oxon for summation. The observed percent inhibition for each of the pairs was compared to the expected percent inhibition obtained from the dose additive model. With brain preparations, the ChE inhibition observed at these low concentrations was typically similar to what would be predicted by this dose-additive model when the two compounds were added simultaneously or when the two compounds were added sequentially. More advanced mathematical modeling strategies, using ordinary differential equations, with calibrations based on the inhibition curves of the individual compounds for brain ChE, have successfully predicted the inhibition resulting from the binary mixtures. In contrast to the brain observations, the serum ChE inhibition was less readily predictable by this dose-additive model, even at very low concentrations, particularly when one of the compounds in the binary mixture was a potent carboxylesterase inhibitor, such as paraoxon. These data suggest that both anti-ChE potency and likelihood for being detoxified will need to be considered in predictive modeling of serum ChE inhibition by organophosphate mixtures. (Supported by American Chemistry Council CRAM 2a-99).

172 DEVELOPMENT OF A RESPONSE SURFACE FOR THE TOXICOLOGICAL INTERACTION BETWEEN CHLORPYRIFOS AND PARATHION USING PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING.

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Chlorpyrifos and parathion are commonly used organophosphate insecticides in agricultural, household and commercial settings. In a biological system, each of these pesticides are activated by phase I (P450) reactions to their respective active forms, chlorpyrifos-oxon and paraoxon. These metabolites are potent inhibitors of the neural acetylcholinesterase. Thus, interactions between these pesticides can occur at two levels in the organism: a) the P450 enzymatic bioactivation site, and b) acetylcholinesterase binding sites. The objective of this study was to develop a physiologically based pharmacokinetic (PBPK) model to predict the joint toxicity of these chemicals in the rat. Model parameters were either calculated, obtained from published animal studies, or optimized using available data. PBPK models were developed for each chemical and its respective metabolite. The metabolite concentrations in blood were then linked to an additional model describing the kinetics of acetylcholinesterase inhibition and aging. The developed overall model was used to investigate the interactions between the parent chemicals and their metabolites with their respective biological sites. A response surface was constructed by using the overall model to simulate the response (percent of inhibited acetylcholinesterase) as a function of dose of each single chemical or various binary mixture combinations. For instance, using an oral dose of 3m/kg of each chemical, the model predicted maximum acetylcholinesterase activity inhibition at 35% and 20% of its initial level by parathion and chlorpyrifos, respectively. Although parathion produced a larger maximum inhibition, chlorpyrifos produced a longer acetylcholinesterase inhibition profile. This study illustrates the use of models to analyze toxicological interactions at dose levels that cannot be tested experimentally.

173 SARIN-INDUCED NEUROTOXICITY AND INCREASE IN BLOOD BRAIN BARRIER (BBB) PERMEABILITY ARE AUGMENTED BY STRESS AND COMBINED EXPOSURE TO PYRIDOSTIGMINE BROMIDE (PB), DEET, AND PERMENTHRIN IN RATS.

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We studied the effect of 28 daily chemical exposures to PB (1.3 mg/kg, po, in water), DEET (40 mg/kg d, in ethanol) and permethrin (0.13 mg/kg, d, in ethanol) and stress by placing the animals in a Plexiglas restrainer for 5 min daily for 28 days on sarin (a single 50 microgram/kg, 0.5 LD50, dose, im, in saline)-induced neurotoxicity. A total of seven groups of rats underwent the following treatments: chemicals, stress, chemicals+stress, sarin, sarin+chemicals, sarin+chemicals+stress, and vehicle control. Brain acetylcholinesterase was inhibited in the cortex, midbrain and brainstem, with sarin+chemicals+stress causing most inhibition. Only sarin+chemicals+stress led to significant inhibition of plasma butyrylcholinesterase. Chemicals and sarin+chemicals increased ligand binding to m2 muscarinic acetylcholine receptor. Treatment with sarin+chemicals+stress increased uptake of [^{3} H]hexamethonium iodide by brain regions in the descending order: midbrain > brainstem > cerebellum > cortex. Increased permeability of BBB was confirmed using anti-EBA monocolonal antibodies and horseradish peroxidase. Histopathological studies with H&E stain showed much greater density of dying (degenerating) neurons in animals treated with sarin+chemicals+stress than other treatments in the cerebral cortex, hippocampus, and cerebellum, where Purkinje cells were more sensitive than molecular cells. Animals treated with sarin+chemicals+stress exhibited greatly enhanced glial fibrillary acidic protein staining, characterized by hypertrophy of astrocytic processes, that correlated with cell death recognizable with H&E stain. These results demonstrate that neurotoxiciy caused by a single sarin exposure is enhanced by combined exposure to stress, alone or with PB, DEET, and permethrin at real-life doses. Supported in part by the US Army Medical Research and Materiel command under contract DAMD 17-98-C-8027.

174 URINARY EXCRETION KINETICS OF TRICHLOROACETIC ACID (TCA) AND DICHLOROACETIC ACID (DCA) IN RATS EXPOSED TO A CHLORINATION BY-PRODUCT (CBP) MIXTURE IN DRINKING WATER.

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Non-invasive biomarkers such as urinary concentrations of parent chemicals and metabolites are potentially useful for exposure assessment. Haloacetic acids, such as TCA and DCA, are potential biomarkers of exposure to CBPs in treated drinking water. However, there has not been an experimental evaluation of their urinary excretion kinetics during controlled exposure to CBP mixtures. The objective of the present study was therefore to characterize the urinary excretion kinetics of TCA and DCA in animals exposed to CBP mixtures. Male Sprague-Dawley rats (180-200 g) were provided *ad libitum* drinking water fortified with a mixture of CBPs (TCA, DCA, monochloroacetic acid, chloroform, bromoform, bromodichloromethane and dibromochloromethane) at two exposure levels for 21 days. The low exposure group received drinking water in which the CBP concentrations were similar to the highest concentrations reported in a Canadian drinking water survey, whereas the high exposure group received water fortified with ten times the highest reported environmental concentration of each CBP. Daily body weight of the rats, water consumed and the urinary output were quantified. The daily, composite urine samples from individual animals were analysed for TCA and DCA using a gas chromatograph with an electron capture detector. For the low

dose group, the daily urinary excretion of TCA varied from 0.043-0.56 mg/L and that of DCA ranged between 0.001-0.025 mg/L, whereas these ranges were 0.84-4.1 mg/L and 0.025-0.25 mg/L, respectively, for TCA and DCA in the high exposure groups. The average % dose eliminated in urine during the experimental period was 60 for TCA and 2.2 for DCA, for the low dose exposures. This controlled exposure study conducted with the rodents suggests that urinary TCA may be developed as a useful biomarker of low-level exposure to CBP mixtures.

175 A BINARY INTERACTION-BASED ALGORITHM FOR PREDICTING THE INTERNAL DOSE OF VOLATILE ORGANIC CHEMICALS (VOCS) IN MIXTURES.

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The objective of this study was to develop and validate a binary interaction-based algorithm for predicting the blood concentrations of VOCs in mixtures. The approach involved the calculation of the blood concentration at steady-state of one chemical (Cs) in the presence of other chemicals competing for hepatic metabolism. The following algorithm was developed: Cs = Cs-ind*(1 + Σ[Am*Ci/(K+Ci)], where Cs = concentration of substrate in the mixture, Cs-ind = concentration of substrate when present individually, Ci = concentration of the inhibitors in the mixture, Am = maximal augmentation of Cs possible in the mixture, and K = inhibition constant specific for each inhibitor-substrate combination. Cs-ind was estimated from steady-state algorithms derived from physiologically-based pharmacokinetic (PBPK) models. Both Am and K were derived from mechanistic studies with each binary mixture. Knowing the Ci measured during mixed exposures, the Cs was calculated on the basis of information on binary interaction mechanisms. The above algorithm was validated using the hepatic venous blood concentration (Cvl) of benzene (B) found in mixtures along with dichloromethane (D), toluene (T), ethylbenzene (E), and m-xylene (X). The Ci values corresponding to D, T, E and X in mixtures were obtained with a previously validated physiologically-based pharmacokinetic (PBPK) model, and were used to predict the modulation of Cvl for B during mixed exposures. The computed Cvl (μg/L) of B was 1.95 for single chemical exposure whereas it was predicted to increase to 3.97, 13.1, 26.7, 33.0, respectively, in the presence of D, D+T, D+T+E, and D+T+E+X (exposure concentrations: B = 1 ppm, D, T, E, X = 50 ppm). These values are identical to those obtained using the validated mixture PBPK model. The results presented in this study demonstrate the possibility of predicting the blood concentrations of chemicals during mixed exposures from knowledge of binary chemical interactions.

176 HEPATOTOXIC EVALUATION OF THE BINARY INTERACTIONS OF CHLOROFORM ($CHCl_3$) WITH BROMODICHLOROMETHANE (BDCM), CHLORODIBROMOMETHANE (CDBM) AND BROMOFORM ($CHBr_3$).

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Trihalomethanes (THMs) result from chlorination or chloramination of natural organic matter in water. Acute and chronic exposure to individual THMs results in target organ toxicity (hepatic, renal) and carcinogenicity. Since human exposure is to multiple chemicals, toxicity data on mixtures of THMs are needed. Three experiments were conducted to examine the binary interactions of $CHCl_3$ with the other 3 THMs. Each experiment consisted of: an aqueous control group; $CHCl_3$ alone at 0.1, 1.0 or 3.0 mmol/kg/day; either BDCM alone, CDBM alone or $CHBr_3$ alone at 0.1, 1.0 or 3.0 mmol/kg/day; 3 mixture groups of the corresponding $CHCl_3$:THM mixture at a 1:1 mixing ratio and total dosages of 0.1, 1.0 or 3.0 mmol/kg/day; and, 2 mixture groups at total dosages of 1.0 or 3.0 mmol/kg/day and varied mixing ratios (2.7:1 $CHCl_3$:BDCM; 6.5:1 $CHCl_3$:CDBM; 65:1 $CHCl_3$:$CHBr_3$) based on the average seasonal proportions of the THMs at 35 water treatment facilities (Krasner et al., 1989). Female CD-1 mice were gavaged daily for 14 days. Hepatotoxicity was assessed by serum SDH. The experimental SDH means were compared with 95% prediction intervals generated by a threshold additivity model built under an assumption of dose addition (Gennings et al., 1997). For $CHCl_3$:BDCM, the closeness of the predicted response under an additivity model and observed mean values indicated no deviation from dose additivity. For $CHCl_3$:CDBM, deviation from dose additivity was not detected for any dose level at either mixing ratio. For $CHCl_3$:$CHBr_3$ significant departure (antagonism) from dose additivity was detected only at 3.0 mmol/kg/kg of the 1:1 mixture; this result must be viewed with caution as the sample size was small in this group due to mortality. In summary, the present data indicate that the binary interactions among the THMs at these mixture dosages and mixing ratios are either additive or antagonistic but not synergistic.(This abstract may not reflect EPA policy.)

177 MICROBIAL GENOTOXICITY OF MODEL CHLORINATED ALIPHATIC HYDROCARBONS AND BINARY MIXTURES.

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Human exposure typically occurs to complex chemical mixtures. This includes environmental exposures, as well as the various mixtures of chemicals in our food. This study addresses the development of *in vitro* methods for assessing the toxicity of complex environmental mixtures, and is focused on binary mixtures of chlorinated aliphatic hydrocarbons. Trichloroethylene (TCE) is one of the most common groundwater contaminants found near hazardous waste sites. The genotoxicity of these model chemicals and binary mixtures of chemicals was measured in the Salmonella/microsome mutagenicity assay. Chemicals were tested both with and without metabolic activation. TCE and dichloroethane (DCE) were tested as liquids by putting various concentrations of the chemicals in glass petri dishes and putting the dish in tedlar bags with agar plates containing the microorganisms. VC was tested as a gas by filling the tedlar bags containing the agar plates with a predetermined volume of gas ranging from 0 - 50% VC. Neither TCE nor DCE were mutagenic with activation. Vinyl chloride (VC) induced a doubling of revertants at all doses tested, with the maximum response of 341 net TA100 revertants observed at a dose of 50% VC. There were no appreciable differences in the response induced by VC alone and the response induced by binary mixtures of TCE or DCE and VC. The mixture of 30% VC and TCE induced 325 net TA100 revertants. Data from the microbial mutagenicity assay indicate that additive responses were induced by mixtures of VC with non-genotoxic chlorinated aliphatic hydrocarbons. (Supported by NIEHS SBRP Grant No. P42 ES004917 and ATSDR Grant No. TU68450502).

178 ANTIESTROGENIC ACTIVITY OF A RECONSTITUTED MIXTURE OF PCBs IN A HUMAN BREAST-CANCER CELL ASSAY.

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Fourteen individual polychlorinated biphenyls (PCBs) and a reconstituted mixture of the 14 PCBs were tested for their estrogenic and antiestrogenic activity in human breast-cancer cell cultures. The reconstituted mixture contained IUPAC-NOs.: 28, 77, 101, 105, 118, 126, 138, 146, 153, 156, 169, 170, 180, 187, and was prepared to reflect the pattern and concentration of PCBs frequently detected in human breast milk. The assay for estrogenic/antiestrogenic activity, the MCF-7 focus assay, is based on postconfluent cell growth and tissue restructuring measured as the development of multicellular layers or foci. The individual PCBs and the reconstituted mixture were tested at 5 concentrations between 1.6×10^{-9}M and 5×10^{-6}M. Two of the 14 PCBs (Nos.: 101 and 138) were estrogenic; they induced the formation of foci at the highest test concentration. Five of the 14 PCBs (Nos.: 28, 77, 126, 153, and 169) were antiestrogenic; they inhibited the foci induced by 1×10^{-10}M estradiol. The reconstituted mixture was antiestrogenic at the three highest test concentrations. The results are compared and discussed in relation to a published report (Hany et al., 1999, TAP 158, 231-243) in which treatment of female rats with the reconstituted mixture resulted in increased uterine wet weights of their offspring. (Supported by NIH ES049795-01A1 and TUBITAK-NATO)

179 AROMATIC FRACTION OF JET FUEL: DOSE-RESPONSE EVALUATION OF A MIXTURE AND ITS INDIVIDUAL COMPONENTS.

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Individual petroleum products are complex mixtures consisting of hundreds of individual components. The assessment of human health risk is complicated by the lack of toxicity data for the exact mixture of concern, although data are available on related components. Recently, a 90-day oral gavage toxicity study was conducted in rats and mice for the equivalent carbon $(EC)_{>8-16}$ aromatic fraction (AF) of jet fuel, containing some aliphatics (Smith *et al.*, 2000, Toxicol. Sciences. 54(1):396). Using this study, we are developing a reference dose (RfD) for the AF. In order to determine if uncertainty factor (UF) values other than the default of 10 could be used to account for subchronic to chronic extrapolation and uncertainty due to an incomplete database, we investigated which individual compounds and mixtures are relevant to the toxicological evaluation of this fraction and could support the derivation of data-derived UFs. A database was constructed using dose-response data

from published studies of chemicals within the AF including styrene, ethylbenzene, xylene, cumene, 1, 3, 5-, 1, 2, 4- and 1, 2, 3-trimethylbenzene, high flash aromatic naphtha (HFAN), naphthalene, 1- and 2-methylnaphthalene, biphenyl and AF. Dose-response data were adjusted to common dose and response metrics for comparison across endpoints grouped by target organ. Evaluation of the database suggests that components of the fraction can provide insight into the expected toxicological behavior of the fraction (e.g., dose-response relationships were similar for rats across hematological, liver and neurotoxicity endpoints for AF, biphenyl, HFAN and cumene). The availability of data for the AF mixture and for some of its components provides an opportunity to evaluate approaches for assessing the toxicity of petroleum-derived mixtures.

180 INTERACTIVE EFFECTS OF DDE AND METHOXYCHLOR ON HORMONE PRODUCTION IN BASS GONADAL CULTURES.

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Environmental exposures invariably involve more than one chemical. The ability to determine whether chemicals have similar modes of action has important implications for regulating pesticides and for mixture risk assessments. p', p'-DDE (DDE) and methoxychlor (MCL) were tested alone and in combination to assess the similarity of their actions on hormone production in gonadal tissue from large mouth bass, a species whose reproductive fitness has relevance to ecosystem health in Florida. Gonads were harvested from adult female bass (2-3 years of age) during the peak reproductive season, minced, and incubated in culture medium with or without test agents for 48 hours. Duplicates of each treatment were performed in each of three experiments using tissue from a different female. Testosterone was measured in aliquots of culture medium by validated RIA procedures. Dose-response relationships of individual agents were characterized over a 6-log concentration range ($1x10^{-2}$ to $1x10^{4}$ ppb). Both DDE and MCL, tested individually, produced a dose-dependent decrease in testosterone levels. Estradiol levels were unaffected. Mixtures of the agents were tested at concentrations of 0.01, 1, 100, and 10, 000 ppb in culture medium in a fractionated factorial, block treatment design. Statistical tests indicated that of 16 dose combinations tested, 15 were antagonistic and only 1 was additive based on the Loewe additivity model of "no-interaction." DDE and methoxychlor are generally recognized to have similar modes of pesticidal action, similar molecular structures and similar effects on the mammalian endocrine system. These results, however, imply that methoxychlor and p, 'p-DDE inhibit testosterone production *via* different mechanisms in bass ovaries.

181 COMPARATIVE REGULATION OF NRK-52E AND HK-2 KIDNEY CELL DEATH PATHWAYS *IN VITRO* BY ARSENIC (As3+), CADMIUM (Cd2+) AND As X Cd COMBINATIONS.

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Arsenic and cadmium are toxic to renal proximal tubule cells but the single and combined effects of these elements on regulatory processes such as protein phosphorylation, stress protein response, and the caspase system have not been fully studied. The present studies examine the effects of As and Cd alone or in combination over concentrations of 10^{-7}M to 10^{-4}M in the rat NRK-52E and human HK-2 kidney cell lines. Preliminary TUNEL assays under these exposure conditions have shown only a minority of cells die from apoptosis. Hence, western immunoblot assays were conducted using antibodies to phosphorylated tyrosine, caspase-3, heme oxygenase, HSP 25, and HSP 27. Results showed marked increases in tyrosine phosphorylation for single and combined elements at 10^{-4}M concentrations in both cell lines. Increased heme oxygenase levels were observed to either coincide with increased tyrosine phosphorylation and/or to precede it at lower doses of 10^{-6}M or 10^{-5}M. Decreased levels of caspase-3 were generally found to coincide with increased tyrosine phosphorylation at 10^{-4}M concentrations of single and combined elements and at 10^{-5}M As x Cd. In HK-2 cells this was accompanied by decreased levels in HSP 27. In rat NRK-52E cells decreased levels in HSP 25 were observed only for 10^{-4}M of As x Cd. DNA fragmentation assays of NRK-52E cells showed no measurable positive results for apoptosis. Western immunoblot analysis of protein kinase C and tyrosine phosphatase PAC-1 in HK-2 cells showed decreased levels for 10^{-4}M doses of single and combined elements. These studies suggest single and combined mixtures of As and Cd may act in concert to inhibit essential regulatory processes leading to apoptosis and thus lead to cell death primarily *via* necrosis. Oxidative stress may play a role as evidenced by elevated heme oxygenase levels and increased levels in phosphorylated tyrosines found for higher doses of single and combined elements may be due to disruption of metabolic oxidative phosphorylation. (Supported by EPA STAR Grant# 827161 and ES 7299-06).

182 AUTOMATION OF RECOMMENDED DEFAULT METHODOLOGY FOR ANALYSIS OF EXPOSURES TO MIXTURES OF CHEMICALS IN EMERGENCIES.

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Exposures to airborne mixtures of chemicals may lead to additive, synergistic or antagonistic health effects. The objective of this paper is to describe automation of default methodology developed for use in emergency management and safety analysis within the Department of Energy complex. For individual chemicals, exposure conditions are evaluated using concentration limits such as Emergency Response Planning Guidelines (ERPGs) or Temporary Emergency Exposure Limits (TEELs). Hazard indices (e.g., HIi = Ci / Limiti, where Ci is the concentration of chemical "i") are calculated for each chemical. For exposure to mixtures, these HIs are summed unless sufficient toxicologic knowledge is available to indicate otherwise. A sum of 1.0 or less means the limits have not been exceeded. If chemicals are known to exert their toxic effect by different modes of action, they can be treated independently, i.e., their individual HIs must be less than one. Only the HIs for chemicals having the same toxic consequences need be added. To facilitate application of these recommendations for analysis of exposures to specific mixtures, chemicals are classified according to their toxic consequences. This is performed using health code numbers (HCNs) describing toxic effects by target organ for each chemical. Only HIs for chemicals having the same HCNs need be added. An Excel Workbook program has been set up to automatically apply this methodology to a user-supplied mixture of chemicals. The workbook comprises six worksheets, one of which is a lookup tabulation of HCNs and TEELs for over 1400 chemicals. These are accessed *via* the Chemical Abstract Services Registry Number (CASRN) or substitute. Only the "Input" worksheet is accessible to the user. The "Input" worksheet has room for entry of up to thirty chemicals. Individual chemical HI s, and the sums of HI s for chemicals with the same HCNs, are automatically calculated and the results are displayed on the "Output" worksheet. This occurs, after data entry is completed, when the Excel workbook is calculated.

183 ALTERNATIVE 5' SPLICING OF HUMAN MICROSOMAL EPOXIDE HYDROLASE: GENETIC LOCATION AND CHARACTERIZATION OF VARIANT EXONS.

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Tissue-specific, chemical, or developmental regulation of gene expression can be directed by splicing events that result in mRNA transcripts with variant exons 1 under the control of alternative promoters. The human microsomal epoxide hydrolase (mEH) gene, important for the disposition of reactive epoxide moieties, has been reported to employ complex alternative splicing programs associated with exon 1 (which is noncoding). We have further characterized and localized these variant mEH exon 1 sequences by *in silico* analyses against the draft human genome. Each of the alternative exon 1 fragments was localized to chromosome one, in close proximity to the location of the previously characterized human mEH gene transcriptional unit (1q42.1). The alternative exon 1 sequences were positioned at distances ranging from at least 15 kbp to more than 46 kbp 5' of the previously established human mEH exon 1. Several of these mEH alternative exon 1 sequences were revealed to be homologous to a gene encoding the human signal recognition particle 9 kDa protein (SRP). In order to confirm the existence of alternative human mEH exons 1 in a variety of tissues, RNA ligase-mediated Rapid Amplification of cDNA End (RACE) reactions were performed on human liver, kidney, and small intestine samples isolated from five subjects. These RACE experiments verified the utilization of alternative mEH exon 1 sequences, but not those homologous to the SRP. Knowledge of the proximal promoter sequence information linked 5' to each mEH alternative exon 1 sequence will enable functional characterization of their gene regulation effects. (Supported by NIH grant ES-04978 and The Burroughs Wellcome Fund)

184 A ROLE FOR HIC-5 IN FOCAL ADHESION FORMATION AND IN HSP27-MEDIATED ACTIN FILAMENT STABILIZATION.

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Hsp27 is believed to stabilze actin filaments, but the mechanism is unknown. We identified hic-5, a focal adhesion protein and androgen receptor co-activator (ARA55), as an hsp27 binding protein, and have mapped the interaction to the hic-

5 C-terminal LIM4 domain and the hsp27 C-terminal domain. We observed that hic-5 inhibits the thermo-protection provided by hsp27. Hsp27 has been suggested to protect cells from heat by stabilizing actin filaments. We hypothesized that hic-5 inhibits hsp27 protection by interfering with hsp27-mediated actin filament stabilization. We analyzed the effect of hsp27-hic-5 interaction on cell polymerized actin (F-actin) using phalloidin binding assay in COS cells transiently transfected with vector only or hsp27, hic-5, hsp27 + hic-5, or hsp27 + hic-5ΔLIM4 (hic-5 mutant that does not bind hsp27) constructs. Hsp27 transfectants had greater and hic-5 transfectants had less total F-actin compared to vector transfected cells. However, coexpression of hic-5, but not hic-5ΔLIM4, with hsp27 reduced cell F-actin content. Cell fractionation studies in identically-transfected NIH 3T3 cells lysed in buffer containing 0.1% triton X-100 revealed hic-5 in the soluble fraction in hic-5 transfectants, but redistribution of hic-5 to the triton-insoluble fraction (that also contains actin cytoskeletal elements) in hsp27 + hic-5 transfectants. Immuno-staining of murine podocytes stably transfected with vector control, hsp27, or hic-5 demonstrated reduced actin stress fibers and focal adhesions in hic-5 transfectants compared to vector control or hsp27 transfected cells. We conclude that hic-5 inhibits hsp27-mediated actin filament stabilization, hic-5 may interfere with the formation of focal adhesions, and hsp27 can regulate the intracellular distribution of hic-5. Supported by NIH grants R01-ES06265, R01-ES07006 and P01-ES11188 to MJW, and K08-DK02455, R01-DK55602 and a research grant from the National Kidney Foundation of Michigan to WES.

185 EFFECTS OF OVEREXPRESSED WILD-TYPE, ATP BINDING- AND ATP HYDROLYSIS-DEFECTIVE YEAST *RAD54* ON DSB-INDUCED CELL KILLING, HR AND CHROMOSOME LOSS.

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DNA double-strand breaks (**DSBs**) in yeast are repaired primarily by homologous recombination (**HR**) involving several proteins including Rad51 and Rad54 which interact during heteroduplex DNA formation. Rad54 alters DNA double helix conformation in an ATP-dependent manner. We examined DSB-induced cell killing, HR, and chromosome loss in **a/α** and **a/a** diploid yeast with normal Rad54 levels, overexpressed wild-type Rad54, or overexpressed mutant versions of Rad54 defective in ATP binding or ATP hydrolysis. DSBs were introduced into *ura3* by HO nuclease. The Heyer Lab. showed that excess wild-type Rad54 reduced methylmethane sulfonate (**MMS**) resistance by 25%, while excess ATP binding or hydrolysis mutant Rad54 reduced MMS resistance by >99%. In contrast, DSB-dependent cell killing with excess wild-type or mutant Rad54 was minimal in **a/α** cells, and modest (25%) in **a/a** cells. MMS is an alkylating agent and its cytotoxic effects are probably replication-dependent, so the different effects of excess Rad54 with MMS and DSB damage may reflect differences in Rad54 function during replication (i.e., during lesion bypass at blocked replication forks by sister chromatid exchange) *vs.* replication-independent DSB repair. In **a/α** and **a/a** cells, excess wild-type Rad54 had no effect on frequencies of DSB-induced HR or chromosome loss, but excess mutant Rad54 reduced HR by 1.5- to 3-fold. In all cases, reduced HR correlated with increased chromosome loss. We reasoned that excess wild-type Rad54 might enhance Rad51-mediated heteroduplex formation and increase gene conversion tract lengths, but the opposite was observed; excess mutant Rad54 had no effect on tract lengths. Together these results suggest that excess wild-type Rad54 does not affect HR initiation but negatively affects the later step of heteroduplex formation, whereas excess mutant Rad54 blocks HR initiation (leading to chromosome loss and some cell killing) but does not affect later steps.

186 CHARACTERIZATION OF INTRINSICALLY THERMOTOLERANT (IT) SUBSTRAINS OF *C. ELEGANS* WHICH MAINTAIN THE IT STATE, SHOW INCREASED LONGEVITY, AND DISPLAY ELEVATED HSP16 LEVELS.

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The classic heat shock or stress response has been studied in a number of organisms including the nematode *C. elegans*. We have previously demonstrated that elevated temperatures as well as exposure to the mitochondrial toxicant sodium azide have the ability to induce a stress response in this nematode, and that this response involves induction of both hsp70 and hsp16. Now, we have isolated substrains of the N2 wild type nematode which show intrinsic thermotolerance, defined as a significantly increased survival probability compared to controls when taken from either 16° or 25° C directly to 37° C for two hours. These N2ITs have maintained their intrinsic thermotolerance for well over two years, and in addition, show a significantly increased life span. Experiments have shown that the average lifespan for worms from the N2IT6 substrain grown at 25 °C is 14.5 ± 0.7 days, compared to 12.5 ± 0.5 days for N2 controls. This represents a 16% increase in average lifespan for this substrain. It was also noted that in every experiment the life span of at least 2-3 N2IT6 worms was significantly longer than the mean (typically on the order of 19 - 20 days). In terms of stress protein expression, Western blot analysis of the N2IT6 substrain indicates elevated hsp16 levels when compared to controls. To further characterize the IT state, we have designed primers and initiated RT-PCR studies from both N2IT6 and N2 controls to determine hsp70, hsp60 and hsp90 expression in these worms. In summary, we have been able to maintain N2IT substrains for over 2 years, and have discovered that these populations show significantly increased longevity compared to N2 controls. In addition, we have demonstrated that these IT substrains show elevated levels of at least one stress protein, hsp16, when compared to N2 controls. We are continuing to investigate the molecular mechanisms by which this IT state is maintained through application of RT-PCR techniques.

187 SULFUR AMINO ACID DEPRIVATION INDUCES MICROSOMAL EPOXIDE HYDROLASE, A PUTATIVE CELL SURFACE AUTOANTIGEN, IN PARALLEL WITH APOPTOTIC CELL DEATH.

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Microsomal epoxide hydrolase (mEH), a detoxifying enzyme and putative cell surface autoantigen, is inducible by xenobiotics and by certain pathophysiological conditions (e.g. tumorigenesis and protein-calorie malnutrition). The present study was designed to determine mEH expression in H4IIE cells during apoptosis initiated by sulfur amino acid deprivation (SAAD) and to identify the signaling pathway for the enzyme induction. SAAD induced apoptotic cell death at 48-72 h and decreased mitochondrial permeability with cytochrome *c* release, both of which were prevented by SB203580 or by dominant-negative JNK1 [JNK1(-)] transfection. Neither caspase-3 activity nor poly(ADP-ribose) polymerase cleavage increased during SAAD-induced apoptosis, indicating that SAAD induced apoptosis independent of caspase activation. This was supported by the observation that benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone, a general caspase inhibitor, did not prevent apoptosis. The levels of mEH mRNA and protein were notably increased in cells under SAAD for 48-72 h. The induction of mEH occurred in parallel with apoptotic cell death. Whereas SAAD-induced apoptosis resulted from both JNK1 and p38 kinase activation, mEH induction was decreased only by JNK1(-) transfection. Immunocytochemistry revealed that mEH protein was intensely stained in apoptotic cells, cellular fragments and cell debris. The number of cells positive for surface mEH also substantially increased by SAAD, as evidenced by flow cytometry analysis. These results demonstrated that SAAD induced apoptosis with an increase in cytochrome *c* release from mitochondria, but not through caspase-3 activation, and that apoptosis induced by SAAD accompanied mEH induction, which involves JNK1, but not ERK1/2 or p38 kinase pathway. The expression of cell surface mEH by SAAD may be associated with presentation of autoantigen.

188 COVALENT BINDING OF ACTIN IS REQUIRED FOR NUCLEAR TRANSLOCATION OF NF-E2-RELATED FACTOR 2 (NRF2) AND ANTIOXIDANT RESPONSE ELEMENT (ARE) ACTIVATION.

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Expression of phase II detoxifying genes is regulated by Nrf2-mediated activation of ARE. Phosphatidylinositol 3-kinase is involved in the translocation of cytoplasmic Nrf2 into the nucleus through rearrangements of actin in response to tert-butylhydroquinone (t-BHQ). The apparent molecular weights of cytoplasmic and nuclear proteins cross-reacting with anti-Nrf2 antibody varies according to reducing condition. The present study was initiated to determine whether Nrf2 could covalently bind with actin. The apparent molecular weight of Nrf2 was 57 kDa in both cytoplasmic and nuclear proteins fractionated from cell lysates prepared in the presence of dithiothreitol, whereas in the absence of dithiothreitol, that of the nuclear protein immunochemically reacting with anti-Nrf2 antibody was 100 kDa. The band intensity of the nuclear 100 kDa protein was increased by t-BHQ treatment. Nuclear translocation of cytoplasmic Nrf2 by t-BHQ was prevented by pretreatment of cells with dithiothreitol. Hence, it is highly likely that Nrf2 translocates into the nucleus as a complex covalently bound with a 43 kDa protein.

Western blot analysis revealed that anti-actin antibody recognized a 100 kDa nuclear protein as well as 43 kDa actin protein. The level of the 100 kDa nuclear protein, which was co-immunoprecipitated with anti-Nrf2 antibody and immunoreacting with anti-actin antibody, increased in cells treated with t-BHQ. Also, the ARE band intensity was reduced in gel-shift analysis after immunodepletion of the nuclear extract with anti-actin or anti-Nrf2 antibody. Addition of dithiothreitol and/or beta-mercaptoethanol to the nuclear fraction failed to convert the 100 kDa protein to 57 kDa Nrf2. Hence, it is unlikely that Nrf2 binds with actin through disulfide bondage. These results demonstrated that the altered cellular redox-state induced by oxidative stress causes Nrf2 to bind with actin and the actin-bound Nrf2 translocates into the nucleus prior to its ARE activation.

189 DEVELOPMENT AND APPLICATION OF AN ALLELE-SPECIFIC GENOTYPING STRATEGY FOR DETERMINATION OF *FMO2*1* FREQUENCY IN A HISPANIC POPULATION.

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The most common allele for the major mammalian lung isoform of flavin-containing monooxygenase, FMO2, encodes truncated inactive protein due to a C to T transition that results in a premature stop codon. The allele (*hFMO2*1*) encoding full-length protein is absent in all Caucasian and Korean individuals genotyped to date; however, it is present in 26% of African-Americans. We recently demonstrated activity of this enzyme in lung microsomes from a heterozygous organ donor. FMO metabolizes the oxygenation of many nitrogen and sulfur containing drugs/xenobiotics. The present study focused on extension of ethnic genotyping to determine the prevalence of *hFMO2*1* among Hispanics, and the screening of candidate substrates with baculovirus expressed hFMO2.1, to assess the potential relevance of allelic variation. Genomic DNA was isolated from lung, liver or blood (plasma or lymphocytes). Allele-specific forward primers, differing in length by 15 nucleotides and one common reverse primer were designed to produce a 245-bp PCR product corresponding to the allele (*hFMO2*2*) encoding truncated protein, and a 230-bp product corresponding to *hFMO2*1*. Allele-specific PCR products were identified by PAGE. DNA, from 27 individuals was sequenced including samples putatively containing the *hFMO2*1* allele, to obtain genotype verification. To date 40 Hispanic individuals have been genotyped; 5.0% have the *hFMO2*1* allele. Baculovirus expressed hFMO2.1 was active toward substituted thiourea compounds with a small cross-sectional area (≤ 4.3 Å), but was inactive toward 1, 3-diphenyl thiourea (11.2 Å), in assays following NADPH oxidation. Thioacetanilide and thiobenzamide were also good substrates. The presence of the ethnic *hFMO2*1* allele in African American and Hispanic populations indicates the potential for ethnic differences in drug and environmental toxicant metabolism. (Supported by PHS grant HL38650.)

190 APPLICATION OF THE RNA INTERFERENCE ASSAY TO INVESTIGATE THE ROLE OF Sp1 AS A TRANSCRIPTION FACTOR IN BREAST CANCER CELLS.

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Sp1 is a member of the Sp family of transcription factors that play an important role in regulating basal expression of multiple genes through interaction with GC/G-rich promoter sequences. The estrogen receptor α (ERα) interacts with Sp1 protein, and several estrogen (E2)-responsive genes are induced in human breast cancer cells through ligand-dependent activation of ERα/Sp1. This study investigates RNA interference (RNAi) through transcriptional and/or posttranscriptional gene silencing of Sp1 protein in MCF-7 cells as a probe for confirming the role of Sp1 and ERα/Sp1-mediated transactivation. For the proposed studies in subconfluent MCF-7 human breast cancer cells, we have used a double-stranded 21 nucleotide small interfering RNA (siRNA) that is homologous in sequence to the coding region 1011-1032 relative to the start codon of Sp1. Lipofectamine was used for transfection of 0.75 μg siRNA per well. Cells were then harvested after 48 hr, and Sp1 protein was examined in whole cell extracts by Western blot analysis. The results showed that immunoreactive Sp1 protein levels were decreased by 60 - 70%. In parallel studies, MCF-7 cells were transfected with a construct (pSp1) containing a single consensus GC-rich Sp1 binding motif, and reporter gene activity was determined in the presence or absence of cotransfected siRNA. The results showed that siRNA decreased Sp1-dependent transactivation by > 90%, whereas in control experiments using siRNA for lamin B, no effects on Sp1 protein levels or pSp1-dependent activity were observed. The RNAi assay is currently being used to target other Sp1- and ERα/Sp1-dependent genes/promoters to investigate contributions of Sp1 and other Sp proteins as transcription factors. (Supported by NIH CA76636 and ES09106)

191 MULTIPLE HISTONE MODIFICATIONS ARE COUPLED TO REACTIVE OXYGEN SPECIES INDUCED ONCOTIC CELL DEATH.

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The rapid formation of DNA single strand breaks, increases in histone H3 phosphorylation, and the dephosphorylation of histone H2A, precede reactive oxygen species (ROS) induced oncotic cell death in renal proximal tubule epithelial cells (LLC-PK1) [Mol Pharmacol, 60:394-402, 2001]. Consistent with these findings, we now report that ROS, generated following the addition of 2, 3, 5-tris-(glutathion-S-yl)-hydroquinone to LLC-PK1 cells, cause the rapid ribosylation of histones H1>>H3 and H4. Histone ribosylation precedes histone H3 phosphorylation, suggesting that ribosylation of linker H1 in particular, provides subsequent access for the requisite kinase(s) to core histone H3. Moreover, ROS induced histone phosphorylation appears to be restricted to a small subset of nucleosomes that are subject to hyperacetylation/ deacetylation. Thus, triton-acid-urea polyacrylamide gel electrophoresis analysis of nuclear extracts from LLC-PK1 cells treated with the histone deacetylase inhibitor, sodium butyrate, reveals that phosphorylated histone H3 comigrates with hyper- (tetra) acetylated histone H3. The data suggest that putative histone H3 kinases selectively target nucleosomes that are also targets of butyrate sensitive histone deacetylase(s). The kinetics of the phosphorylation/acetylation coupling remains to be determined. Finally, in this model of ROS-induced oncotic cell death we fail to observe any changes in histone methylation. Because methylation of K9 and K27 in histone H3 are suggested to hinder S10 and S28 phosphorylation, the combined data indicate that specific and precise histone modifications occur during ROS induced cell death and that such selective histone modifications are supportive of the "Histone Code" hypothesis. (ES 07247, ES 07784)

192 TETRAFLUOROETHYLCYSTEINE-INDUCED ALTERATIONS TO MITOCHONDRIAL COMPLEX III ACTIVITIES *IN VITRO*.

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The potent nephrotoxicant tetrafluoroethylcysteine (TFEC) induces cell death by initiating damage exclusively within the mitochondrial compartment. Nonetheless, *de novo* transcription of nuclear genes is a well recognized response to TFEC treatment *in vivo* and *in vitro*. Consequently, we examined the possibility of communication from mitochondria to the nucleus, analogous to a well-characterized communication pathway in *Saccharomyces cerevisiae* ("retrograde regulation", RTG). Degenerative RT-PCR of structural motifs of the yeast RTG3 gene reproducibly yielded a 475 bp fragment identical to a segment of the mitochondrial cytochrome *b* component of Complex III (ubiquinol:ferricytochrome *c* oxidoreductase). Alterations to antimycin A-sensitive Complex III activities were examined in an accurate mammalian *in vitro* model for TFEC-induced cell death to determine the biological significance of this finding. TFEC treatment resulted in specific time-dependent decreases in Complex III activity (49.7 ± 0.8 mmol cytochrome *c* reduced/min/mg protein at 12 h v/s 79.2 ± 12.6 mmol/min/mg protein control: mean ± s.d.; n=3, p<0.001). Cell viabilities were also determined by live/dead® stain and morphological alterations by light microscopy. To determine if these changes correlated with a particular mode of cell death, antimycin A-sensitive Complex III activities were also evaluated after treatment with actinomycin D/tumor necrosis factor α, acetaminophen, or other toxicants. These results show a specific effect on Complex III activity by TFEC and suggest a mechanism by which changes to oxidative phosphorylation may be coupled to nuclear transcription *via* alterations to cytochrome *b*. *(Supported by NIH grant #GM25418 to SDN, #GM51916 to SB and P30 ES07033 to the UW Center for Ecogenetics and Environmental Health)*

193 DIFFERENCES IN METHYLATION PATTERNS OF THE ESTROGEN RECEPTOR AND AROMATASE GENES IN JAPANESE MEDAKA.

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In order to explore the potential for environmental contaminants to alter patterns of genetic imprinting, we are determining the tissue and sex specific patterns of methylation in the promoter region of the estrogen receptor and the aromatase genes of the Japanese medaka, *Oryzias latipes*. Primers were designed to span the CpG islands of the medaka promoter region where there were suspected sites of

methylation. Livers, gonads, and brains were removed from male and female medaka and the genomic DNA extracted. Samples of genomic DNA from each sex and tissue were treated with sodium bisulfite (resulting in a loss of all non-methylated cytosine residues), amplified, cloned, sequenced and compared to the genomic sequence. Cytosine residues remaining in the bisulfite-treated samples were from methylated CpG islands. In the estrogen receptor, the female liver, brain and ovary had 13 and the male liver had 15 CpG islands in the promoter respectively, all of which were methylated. The male gonad and brain only had 12 and 13 CpG islands respectively, 11 of which were methylated. Because the overwhelming majority of the CpG islands showed methylation, we had speculated that one possible mechanism for increase in transcription rates of the estrogen receptor was through a loss of methylation at these sites. However, unexpectedly, the remaining three non-methylated islands (1 from gonad and 2 from brain) were methylated in fish exposed to 500ng/L ethynylestradiol, but not in control males. In contrast to the estrogen receptor, the aromatase gene had only 5 CpG islands in the promoter region of the wild type male tissues. Further studies will look at the efficacy of other environmental contaminants in altering patterns of methylation in these two genes.

194 BIOCHEMICAL STUDIES ON THE SOLUBLE FRACTION OF HEN PERIPHERAL NERVE: SEARCHING THE TARGET OF PROMOTION OF AXONOPATHIES.

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Promotion of axonopathies is caused by certain esterase inhibitors such as sulfonyl halides, carbamates, thiocarbamates and phosphinates. Promotion was found to correlate with inhibition of a phenyl valerate esterase (PV) activity in the whole nerve homogenate (M200). M200 was defined as the activity resistant to 40 μM paraoxon and with an IC50 for mipafox of about 200 μM. Sephacryl S-300 chromatography of soluble proteins of peripheral nerve separated most PV activity (S-PV)in fractions corresponding to 50-70 kDa MW proteins. S-PV was found to be selectively inhibited by promoters after *in vivo* treatment with several esterase inhibitors. At least 3 proteins, as seen from SDS-PAGE, were present in such fractions. It has been reported that S-PV activities are reversibly inhibited by paraoxon (Barril and Vilanova, 1999). In fact, when S-PV activity was inhibited (77%) by paraoxon (2 μM, 20 min, 37 °C, pH 8.0), then a 78% reversibility was observed after 24 hours dialysis at 0 °C. Consequently, the S-PV IC50s for mipafox were higher in the presence of paraoxon. S-PV activity found to be sensitive to mipafox was about 90% of total, whereas that sensitive to tested inhibitors varied from 70 to 90%. The IC50s for mipafox were 20, 50 and >100 μM in the presence of 1, 8 and 40 μM paraoxon, respectively, and 1 μM in the absence. Similarly, the S-PV IC50s for several promoters were found to be lower than those of M200. These were: for PMSF 1 and 60 μM, for L(-) methamidophos 200 μM and >20 mM, for D(+) methamidophos 400 μM and 5 mM, for n-hexyl methamidophos <1 and 100 μM, and for molinate sulfone 0.01 and 1μM, respectively. In conclusion, S-PV activity displays *in vitro* sensitivity to promoters higher than that of M200. This is due to different assays which are required for either M200 or S-PV, because of the reversible inhibition of S-PV caused by paraoxon.

195 ACTIVATION OF NUCLEAR FACTOR-kappaB (NF-KB) BY CIGARETTE SMOKE CONDENSATE.

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Cigarette smoking is a major risk factor for lung cancer and coronary artery disease. Pro-oxidant constituents of tobacco smoke have been implicated in smoking-associated disease development because of their potential role in inducing oxidative stress. We postulated that activation of redox sensitive transcription factor, NF-KB, may play a role in tobacco smoke-induced cell damage. To test this hypothesis, activation of NF-KB was examined in cigarette smoke condensate (CSC)-treated human monocytic cells (U937). The cells were treated with CSC in serum free medium for 30 min. Nuclear and cytoplasmic extracts were prepared and analyzed for NF-KB binding by EMSA and I-KBαcontent by immunoblots, respectively. Parallel experiments were carried out with TNF-α as positive control. The results showed that treatment of cells with CSC increased NF-KB -DNA binding in a dose and time dependent fashion. Analysis of the cytoplasmic extracts showed that I-KBαlevels started declining with in 15 mins of CSC treatment of cells and were significantly diminished by 60 mins. Further experiments showed that IKBαkinase was activated in cells with in 15 min of treatment with CSC. These observations indicated a potential of CSC to initiate phosphorylation and degradation of IKBα. Tests with nuclear extracts of various other CSC-treated cells of lymphoid and epithelial origin also showed increased NF-KB-DNA binding thus suggesting that CSC-mediated NF-KB activation is not cell specific. It is concluded that NF-KB activation by CSC may mediate tobacco smoke-induced cell damage (supported by NIH (P01 CA91844); Institutional Tobacco Funds, and KTRB-5-41165).

196 DUAL FUNCTIONS OF YEAST DNA POLYMERASE ζ IN DNA LESION BYPASS.

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Saccharomyces cerevisiae DNA polymerase ζ (Polζ) is required for DNA lesion bypass during replication. To gain insights into the function of Polζ in lesion bypass, we have performed *in vitro* biochemical analyses of this polymerase in response to several DNA lesions. Purified yeast Polζ performed limited translesion synthesis opposite a template (+)-trans-anti-BENZO(A)PYRENE-N^2-dG adduct and a template (-)-trans-anti-BENZO(A)PYRENE-N^2-dG adduct by preferentially incorporating G, and less frequently C. Opposite an acetylaminofluorene (AAF)-adducted guanine, purified yeast Polζ predominantly incorporated a G. This lesion, however, significantly inhibited subsequent extension DNA synthesis. Extension synthesis by yeast Polζ was more efficient when A or C was opposite the AAF-guanine. These results support the following dual-function model of Polζ. First, Polζ catalyzes nucleotide incorporation opposite (±)-trans-anti-BENZO(A)PYRENE-N^2-dG adducts and AAF-guanine with a limited efficiency. Secondly, more efficient bypass of these lesions may require nucleotide incorporation by other DNA polymerases followed by extension DNA synthesis by Polζ.

197 *IN VIVO* EXPOSURE TO IMMUNOTOXIC DOSES OF BENZO(A)PYRENE INDUCES CYTOCHROME P450 1A EXPRESSION WITHIN KIDNEY MONONUCLEAR CELLS OF FISH.

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BENZO(A)PYRENE (BaP) is a widespread environmental contaminant and known carcinogen. In addition to its cancer promoting ability, BaP also has profound effects upon mammalian immunocompetence. Previous studies conducted in this laboratory have utilized a small teleost fish species, Japanese medaka (*Oryzias latipes*), as a laboratory model for investigating the immunotoxic potential of BaP. Similar to that observed in rodents, immune responses of medaka were suppressed following IP injection of BaP at doses that failed to affect morbidity or host survival. Specifically, BaP dose-dependently reduced antibody-forming cell (AFC) numbers. Furthermore, co-exposure to compounds that inhibit hepatic CYP1A activity ameliorated the immunotoxicity produced by exposure to BaP alone. This suggests that BaP metabolism into reactive metabolites may be required for suppression of immune function. Further studies in this laboratory have revealed that injection of BaP, at the same concentrations that suppressed AFC number, induced expression of CYP1A within a distinct subpopulation of kidney (primary site of hematopoiesis / leukopoiesis in medaka) mononuclear cells. Isolation of mononuclear cells from the kidney following *in vivo* exposure to BaP resulted in immunopositive staining of CYP1A within a small percentage of both glass-adherent and non-adherent immune cell types; little CYP1A expression was seen in isolated cells from the vehicle control. Moreover, exposure of isolated kidney immune cells to BaP *in vitro* resulted in both an induction of CYP1A expression and a concurrent decrease in AFC number following *in vitro* immunization. This later finding suggests that specific medaka immune cells are sensitive to the parent BaP compound. Since it is apparent that medaka immune cells possess inducible CYP1A, it is possible that BaP may directly alter the function of these cells *via* the production of immunotoxic BaP metabolites *in situ*. (DAMD 17-99-9011and 60-1-8109)

198 EFFECT OF RETINOIC ACID ON 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)-INDUCED MATRIX METALLOPROTEINASE EXPRESSION IN HUMAN KERATINOCYTES.

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Exposure to the environmental contaminant 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) results in an increased incidence of skin cancers. TCDD-exposure results in activation of the AhR/Arnt signaling pathway, mediating changes in gene expression through binding to XREs (Xenobiotic Response Elements). The AhR/Arnt pathway also interacts and influences other signaling pathways, including the retinoic acid (RA) pathway. Retinoids are powerful regulators of cell growth and differentiation and are widely used in the prevention and treatment of a variety of cancers in humans. Preliminary data from our laboratory suggests that interaction of these pathways alters the expression of matrix metalloproteinases (MMPs), proteases involved in tumor invasion. MMPs degrade the protein components of the extracellular matrix and the basement membrane, which is required for tumor cell migration and invasion. Northern analysis demonstrates that TCDD exposure of normal human keratinocytes results in activation of MMP expression, and that co-treatment with RA further stimulates MMP expression. The mechanism of activation of MMP-1 expression by TCDD + RA may involve binding to AP-1 sites in

the proximal promoter, or potential XREs in the distal part of the promoter. Mobility shift data demonstrates TCDD activation of binding to the AP-1 site and to the XREs following TCDD treatment. Binding to a consensus XRE is abolished following RA exposure, whereas binding to the AP-1 site appears unchanged. Future experiments will examine the role of these elements in TCDD-RA induction of MMP expression, and to determine the effect of TCDD-RA treatment on cellular migration and invasion.

199 TCDD STIMULATES ANDROGEN-RECEPTOR SIGNALLING IN LNCAP CELLS.

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TCDD, the most extensively studied aromatic hydrocarbon receptor (AHR), has a high affinity for the AHR and exhibits a broad range of toxic, biological effects in humans and laboratory animals, including tumor promotion, lymphoid involution, embryonic toxicity, epithelial hyperplasia and metaplasia, wasting syndrome, carcinogenesis, and endocrine disruption. Several mechanisms have been proposed to explain how AHR activation by TCDD interferes with steroid-regulated functions. These mechanisms include: (1) enhancement of ligand metabolism, (2) alteration of hormone synthesis, (3) down-regulation of receptor levels, and (4) interference with hormone-induced gene transcription and cell proliferation. In male rats exposed to TCDD, decreased growth of the testis, epididymis, and prostate were observed. These effects did not correlate with decreases in circulating androgen or androgen receptor (AR) levels, suggesting that TCDD does not interfere with androgen synthesis or metabolism, or with the levels of AR. Therefore, we hypothesized that TCDD interferes with AR-mediated gene expression and cell proliferation. To test this hypothesis, we utilized androgen-dependent human Lymph Node Cancer of the Prostate (LNCaP) cells to determine the effects of TCDD on androgen-regulated functions. Our studies indicate that TCDD stimulates cell proliferation in LNCaP and activates gene transcription of the human PSA (prostate specific antigen) gene promoter and of the MMTV (mouse mammary tumor virus) LTR in LNCaP as well as in African green monkey CV-1 cells, which lack AHR expression. These results point to a possible role of environmental toxicants in the androgen-independent progression of prostate cancer. Supported by NIH Grants 2RO1 ES06273 and ES05941-02.

200 DEC1 BASIC HELIX-LOOP-HELIX DIFFERENTIALLY REGULATES GENES ENCODING CARBOXYLESTERASES AND CYTOCHROME P450 ENZYMES.

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DEC/STRA/SHARPs represent a new and distinct class of basic helix-loop-helix (bHLH) transcription factors. Human DEC1 is abundantly present in several normal tissues including heart, brain, placenta, lung and liver. In order to determine whether DEC1 regulates genes highly expressed in liver, transactivation and transrepresion activity towards several liver gene reporters was determined in stable transfected cell lines expressing DEC1 or a mutant (lacking the DNA binding domain). The expression levels of DEC1 or its mutant were inducibly regulated by tetracycline. The reporters were constructed to include a promoter fragment (~2 kb) from genes encoding drug-metabolizing enzymes such as carboxylesterases and cytochrome P450 (CYP) enzymes. The stable transfected lines were cultured in the presence or absence of tetracycline and then transiently transfected with a promoter reporter and the pRL-TK plasmid (transfection efficiency control). After a 24-hr incubation, the reporter activities were determined with a dual luciferase system. The activity from cells cultured in the presence of tetracycline was used to compare with the activity of cells cultured in the absence of this antibiotic. The ratio of two values was used as an indicator of DEC1-mediated effect. Among ten reporters used, four reporters including CYP2C8, 9, 19 and 3A4 were markedly repressed by DEC1 (~50%). The repression was observed only in cell line expressing full-length DEC1 but not the mutant, suggesting that repression of these reporters requires DNA binding. Factors such as cAMP and hypoxia are known to cause up-regulation of DEC1. In rats these factors are shown to down-regulate several CYP enzymes. The data presented likely provide a novel mechanism regarding the suppression of liver specific gene expression.

201 SELECTIVE MODIFICATION OF CYS186 IN RAT LIVER CARBONIC ANHYDRASE III BY ACRYLONITIRLE *IN VIVO*.

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Covalent binding of reactive chemical species to tissue proteins is a common, but poorly understood, mechanism of toxicity. Identification of the proteins and the specific amino acid residues within the proteins that are chemically modified will aid our understanding of the toxification/detoxification mechanisms involved in covalent binding. Acrylonitrile (AN) is a commercial vinyl monomer that is acutely toxic and readily binds to tissue proteins. Total covalent binding of acrylonitrile to tissue proteins is highly correlated with acute toxicity. Two-dimensional polyacrylamide gel electrophoresis and autoradiography were used to locate proteins in male rat liver cytosol that are radiolabeled following administration of [^{14}C]AN *in vivo*. Five intensely labeled proteins of approximately 30 kD were prominent in the autoradiogram. Tryptic peptide mapping by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry was used to identify all five of the proteins as carbonic anhydrase III (CA III). HPLC of the tryptic digests combined with MALDI-TOF mass spectrometry were used to localize the radiolabel to tryptic fragment T22 containing amino acids 171-187. This tryptic fragment contains two cysteine residues (Cys181 & Cys186) in the rat CA III sequence. However, using MALDI-TOF mass spectrometry, we were not able to determine which of the two residues is modified by AN. Electrospray ionization ion-trap mass spectrometry was used to sequence the peptide and establish that only Cys186 is labeled. Thus, although AN is considered to be highly reactive, our data indicate that it does not react indiscriminately with rat CA III but rather is selective for one out of five cysteine residues, namely Cys186. Rat liver CA III has previously been shown to protect cells against oxidative stress. These data suggest that CA III is also capable of scavenging reactive xenobiotics and may help prevent covalent binding to more critical macromolecules. Supported by NIEHS ES06141.

202 THE N-TERMINAL REGION OF RAT PREGNANE X RECEPTOR POTENTIATES THE TRANSACTIVATION OF THE WILD-TYPE RECEPTOR AND COREPRESSOR SUN REVERSES THE POTENTIATION EFFECT.

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The pregnane X receptor (PXR) is shown to interact with a wide array of dissimilar compounds and confer induction of cytochrome P450 3A (CYP3A) enzymes. The PXR is structurally divided into a DNA binding domain, a ligand binding domain and an N-terminal uncharacterized sequence. We recently isolated from a rat liver library a mutant PXR cDNA designated rPXR-2. This cDNA had an insertion in the region encoding the DNA binding domain. As a result, rPXR-2 produced at least two polypeptides: the N-terminal uncharacterized and the incomplete C-terminal ligand binding domain. Co-transfection experiments demonstrated that rPXR-2 showed no transactivation activity toward a CYP3A reporter. However, rPXR-2 was shown to potentiate the transactivation activity of the wild-type PXR (rPXR-1). Dissecting experiments showed that the N-terminal uncharacterized sequence had the potentiation activity. Such a potentiation effect, however, was reversed by corepressor SUN. These findings suggest that suggests that PXR transcription complex contains a co-repressor and increased truncated PXR levels deplete the co-repressor pool, which represents a novel signaling mechanism in regulating the expression of genes encoding drug-metabolizing enzymes.

203 1, 2-DICHLOROBENZENE MEDIATES THE PRODUCTION AND RELEASE OF FACTORS IN HEPATOCYTES THAT ENHANCE KUPFFER CELL ACTIVITY.

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1, 2-Dichlorobenzene (1, 2-DCB), a potent hepatotoxicant in Fischer-344 (F-344) rats, produces hepatocellular oxidative stress as a result of 1, 2-DCB bioactivation followed by a second oxidative burst mediated by activated Kupffer cells (KC). KC activation is responsible for the extensive hepatocellular necrosis associated with 1, 2-DCB. The hypothesis of this work is that KC activation, in part, is caused by cytokines and/or chemokines produced and released from compromised hepatocytes. Incubation of hepatocytes with 1, 2-DCB (3.6 umol) enhanced the production and release of cytokine induced neutrophil chemoattractant factor (CINC) by 2.2-fold and increased mRNA expression of macrophage migration inhibitory factor. These two proteins may activate KC. The production and release of CINC was not observed in hepatocytes incubated with 1, 4-dichlorobenzene (a nonhepatotoxic isomer) or L-buthionine sulfoxamine (to deplete glutathione). Interestingly, the receptor for CINC, CXC Receptor 2, was expressed on KC as determined by immunohistochemical analysis. When conditioned media from 1, 2-DCB (3.6 umol) treated hepatocytes was incubated with KC these macrophages had increased transcription factor NF-kB binding activity (4.6-fold greater than control) and enhanced release of nitric oxide (21% above control). Collectively, these data suggest that 1, 2-DCB triggers a cascade of molecular processes in hepatocytes that promote the expression and release of oxidant sensitive chemokines. These products signal the activation of KC and the upregulation of the inflammatory response leading to the progression of 1, 2-DCB induced liver damage. (Supported in part by NIEHS Grant (ES06094), The Coca-Cola Foundation and an AFPE Fellowship).

204 DIORTHO CHLORINE SUBSTITUTED PCB CONGENERS ACTIVATE MITOGEN ACTIVATED PROTEIN KINASES INDEPENDENT OF PROTEIN KINASE C ACTIVATION IN JB6 MOUSE EPIDERMAL CELLS.

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Polychlorinated biphenyls (PCBs) are widespread environmental contaminants that evoke a variety of toxic effects including carcinogenicity in experimental models. The co-planar PCB congeners bind with the Ah receptor (AhR) and their toxic effects are most likely mediated through the AhR. The di-ortho chlorinated non-coplanar PCBs congeners do not bind with the AhR. Their toxic effects may, therefore, occur independent of AhR. The mechanisms of their toxic interactions remain to be elucidated. In this study we examined the potential of two non-coplanar PCBs 2, 2', 4, 4'-tetrachlorobiphenyl (TCB) and 2, 2', 4, 4', 5, 5'-hexachlorobiphenyl (HCB) to activate mitogen activated protein kinase (MAPK) cell signaling cascade in JB6 mouse epidermal cells. Activation of the extracellular signal regulated kinase (ERK) group of MAPKs in JB6 cells in response to PCB exposure was assessed by western blotting using phosphorylation state specific antibodies. Treatment of the cells for one hour with 20 μM of either of these PCBs caused a six- to eight-fold increase in the activation state of the ERK-MAPKs. The activation of ERKs by these PCBs was also dose dependent and sustained for more than 12 hrs. Pre-treatment of JB6 cells with a protein kinase C (PKC) inhibitor bisindolylmaleimide (5.0 μM) attenuated ERK activation by a potent PKC activator 12-tetradecanoyl phorbol 13-acetate but not that by the PCBs. These data suggest that ortho chlorinated PCBs may cause epigenetic alterations through activation of ERK-MAPKs independent of PKC activation (supported by NIEHS Superfund grant no. ES04911).

205 THE AH RECEPTOR AND AH RECEPTOR NUCLEAR TRANSLOCATOR MEDIATE STIMULATION BY OLTIPRAZ OF THE XENOBIOTIC RESPONSE ELEMENT IN THE RAT UGT1A6 GENE.

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The protective action of the chemopreventive drug, oltipraz (OTP), has been attributed in part to its selective inducing effects on phase 2 detoxifying enzymes such as glutathione-S-transferase (GST), NAD(P)H quinone oxidoreductase (NQO), and UDP-glucuronosyltransferase (UGT) isoforms 1A6 and 1A7. The mechanism of induction of GST and NQO by OTP has been reported to be mediated transcriptionally *via* antioxidant response elements (ARE), but this has not been established for UGT1A6. Previously we reported that OTP (50 μM) stimulated expression of a transfected UGT1A6-luciferase reporter plasmid in primary rat hepatocytes (PRH) and HepG2 human hepatoma cells. This effect required an intact xenobiotic-response element (XRE) located between bases -134 and -129. To confirm the role of the XRE in OTP inducibility, pGL3-promoter constructs containing 1 to 3-copies of the UGT1A6 XRE conferred responsiveness to OTP in PRH. OTP induced CYP1A mRNA as well as transfected CYP1A reporter genes, indirectly supporting the ability of OTP to induce genes under XRE control. Two lines of direct evidence support the involvement of the arylhydrocarbon receptor (AHR) and AHR nuclear translocator (ARNT) in mediating the effect of OTP on the UGT1A6 XRE. Cotransfection with AHR or ARNT expression plasmids enhanced UGT1A6 reporter plasmid induction by OTP. Electrophoretic mobility shift assays using UGT1A6 XRE oligonucleotide as probe and nuclear extracts from OTP and 3-methylcholanthrene treated HepG2 cells revealed that both agents induce specific complex formation on the UGT1A6 XRE. The specific binding was effectively competed by a CYP1A XRE oligonucleotide but not by a mutant UGT1A6 XRE oligonucleotide. Complexes were supershifted by antibodies to both AHR and ARNT. These results provide the first direct evidence that OTP may also induce biotransformation enzymes *via* an AHR-based mechanism and suggests that OTP transcriptionally activates both phase 1 and phase 2 enzymes. (Supported by ES07762 and ES078087).

206 NRF2-DEPENDENT NAD(P)H:QUINONE OXIDOREDUCTASE EXPRESSION IN MOUSE CORTICAL ASTROCYTES.

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The antioxidant responsive element (ARE) mediates transcriptional regulation of NAD(P)H:quinone oxidoreductase (NQO1). Previously, we demonstrated that ARE activation depends on Nrf2 and that oxidative stress is not involved in tert-butylhydroquinone (tBHQ)-mediated ARE activation in IMR-32 human neuroblastoma cells. In this study, we report that b-zip transcription factor Nrf2 plays a major role in transcriptional activation of NQO1 in mouse cortical astrocytes. For this study, we used primary cortical astrocytes of newborn pups from a Nrf2+/- X Nrf2+/- breeding. Nrf2-/- astrocytes showed markedly decreased basal NQO1 enzymatic activity and no induction by tBHQ compared to Nrf2+/- or Nrf2+/+ astrocytes. In addition, ARE-luciferase reporter gene expression was decreased in Nrf2-/- astrocytes compared to Nrf2+/- or Nrf2+/+ astrocytes in transient transfection experiment. Finally, decreased reporter gene expression was restored by overexpression of wild-type Nrf2 in Nrf2-/- astrocytes, and ARE-luciferase gene expression in Nrf2+/- astrocytes was completely blocked by overexpression of dominant negative Nrf2. Previously we demonstrated Nrf2 nuclear translocation is the critical event for ARE activation in IMR-32 cells. Similarly, tBHQ treatment induced nuclear translocation of Nrf2 in mouse cortical astrocytes. Taken together, these data suggest that both basal and inducible NQO1expression are dependent on Nrf2 in mouse astrocytes. (Funded by ES08089, ES10042, BWF New Investigator Award).

207 INVOLVEMENT OF HEPATOCYTE NUCLEAR FACTOR-1 IN THE REGULATION OF RAT UDP-GLUCURONOSYLTRANSFERASE 1A6 (UGT1A6) GENE.

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UDP-glucuronosyltransferase 1A6 (UGT1A6) facilitates the removal of phenolic compounds from the body, thereby preventing their further metabolism and subsequent toxicity. UGT1A6 exhibits a complex pattern of tissue and inducer-specific expression. A possible basis for this complex pattern was suggested in a previous study identifying a second transcription unit (class 2) giving rise to UGT1A6. The class 2 transcript utilizes an alternative promoter designated "UGT1A6P2" which, on the basis of RT-PCR and RNAse protection data, was found to be highly expressed in liver, kidney, and GI tract relative to other extrahepatic tissues. The observation that an apparent hepatocyte nuclear factor-1 (HNF1)-binding site resides in the region immediately flanking UGT1A6P2 (bases -52 to +38) suggested a possible mechanism for this profile of expression. The current study was carried out to assess the functionality of this putative HNF1 binding site. DNAse I footprinting assays suggested specific binding by nuclear extract from rat liver to this site. This binding was confirmed using electrophoretic mobility shift assays. Nuclear extracts from rat liver and HepG2 cells exhibited high binding activity, whereas Hepa1 extract exhibited no detectable binding. The specificity of binding was confirmed in competition assays. Antibodies to HNF1α and to a lesser extent HNF1β resulted in a visible supershift in mobility of the complex. The functionality of this site in liver is supported by (1) the reduced expression of a UGT1A6P2 reporter in HepG2 cells when the HNF1 site is mutated and (2) the enhanced expression from UGT1A6P2 in both HepG2 and Hepa1 cells when either HNF1α or HNF1β are overexpressed. In contrast, the UGT1A6P1 promoter does not appear to contain a consensus HNF1 site and shows lower responsiveness to overexpression of HNF1α or HNF1β. These data provide a possible basis for the high expression of the UGT1A6 "P2" promoter observed in liver and other tissues known to be enriched in HNF1. (Supported by ES07762 and ES078087).

208 COMPARISONS AMONG PERFLUORINATED COMPOUNDS OF EFFECTS ON GAP JUNCTIONAL INTERCELLULAR COMMUNICATION.

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Gap Junctional Intercellular Communication (GJIC) is the major pathway of intercellular signal transduction, thus is very important for normal cell growth and function. Studies have demonstrated the ability of some perfluorinated organics to distrupt GJIC in *in vitro* cell culture assays. Recent studies have revealed a global distribution of perfluorinated compounds, especially perfluorooctane sulfonic acid (PFOS), in the environment. Therefore, the effects of PFOS, perfluorooctanoic sulfonamide (PFOSA), perfluorohexane sulfonate (PFHS) and perfluorobutylic sulfonate (PFBS) on GJIC were studied using rat liver epithelial (WB-F344) cell line and Carvan dolphin kidney epithelial (CDK) cell line. Effects on GJIC were measured using the scrape loading dye technique. PFOS, PFOSA and PFHS were found to inhibit GJIC in a dose dependent fashion, and this inhibition occurred rapidly and reversibly. EC50s for inhibition of GJIC in rat cells were 29.96, 36.6 and 122 uM (15, 18.3 and 61 mg/L) for PFOS, PFOSA and PFHS respectively. PFBS showed no significant effects of GJIC within the concentration range tested. A structure activity relationship was established based on the four tested perfluoro compounds, and it was indicated that the inhibitory effect was determined by the length of fluorinated tail not the functional group. Since the results from two cell lines were comparable to each other, it was also suggested that the inhibitory effects of selected perfluorinated compounds on GJIC were neither species- nor tissue-specific. The inhibition of GJIC observed in *in vitro* was confirmed in rats exposed to PFOS *in vivo* with a LOEC based on liver accumulation of PFOS of 54 ppm

(108 mg/L). Because of the relatively great concentrations of PFOS accumulated in liver in the *in vivo* experiments determination of NOECs based on tissue concentrations was not possible.

209 DISULFIRAM INDUCES SKELETAL MUSCLE DEGENERATION AND BEHAVIORAL ALTERATIONS IN LONG EVANS RATS.

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Disulfiram is a drug from the family of reactive compounds known as Dithiocarbamates. It is used clinically, industrially and agriculturally. It is established that these compounds promote the chelation of metals, interact with sulfhydral groups, enhance oxidative reactions, inhibit many metal-dependent and sulfhydral enzyme systems and adversely affect the CNS and PNS. Disulfiram has been shown by transmission electron microscopy to cause skeletal muscle degeneration in rats after 9 weeks of oral treatment with 1500 mg/kg, characterized by ultrastructural changes including disintegration and disorientation of myofilaments characterized by fiber splitting and ultimate loss of cross striations, vacuolization and necrosis. In this study, male Long Evans rats treated by oral intubation with Disulfiram at 750 mg/kg in a polyethylene glycol 400 vehicle or treated with vehicle alone, 3x/week were sacrificed at 1, 3 and 6 weeks. Skeletal muscle (vastus medialis) was dissected and fixed in Bouin's fixative followed by 10.0% neutral buffered formalin (pH 7.4). Sections were stained with hematoxylin and eosin (H&E), Mallory's phosphotungstic acid hematoxylin (PTAH) and Masson's trichrome methods. Additional samples were used for determination of malondialdehyde (MDA) and 4-hydroxyalkenals (4-HNE) using a lipid peroxidation assay. Behavioral studies included the open field test and hind-limb splay angle. In all rats given Disulfiram for 6 weeks, a focal subacute myofibrillary degeneration was observed characterized by swollen and disrupted fascicles. Treated animals showed alterations in locomotor activity associated with increased excitability as measured by number of squares traversed and number of rears. Decreased body weight gain as compared to controls was seen. Preliminary results showed no changes in lipid peroxidation among groups.

210 MERCURY INDUCES CYTOTOXICITY, AND TRANSCRIPTIONALLY ACTIVATES STRESS GENES IN HUMAN LIVER CARCINOMA ($HEPG_2$) CELLS.

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Mercury is a non-essential element that exhibits a high degree of toxicity to humans and animals. Exposure to mercury has been associated with a significant number of adverse health effects including: cardiovascular disease, anemia, developmental abnormalities, neurobehavioral disorders, kidney and liver damage, and cancer in some cases. In several studies, the toxicity of mercury has been attributed to its high affinity to protein-containing sulfhydryl groups. However, little is known regarding the molecular mechanisms by which mercury exerts its toxicity, mutagenesis, and carcinogenesis. This research was therefore designed to assess the cellular and molecular responses of human liver carcinoma cells following exposure to mercury. Cytotoxicity was evaluated using the MTT-assay for cell viability, while the gene profile assay was performed to measure the transcriptional activation of stress genes in thirteen different recombinant cell lines generated from HepG2 cells. Cyctotoxicity experiment yielded a LD_{50} value of 3.5 ± 0.6 ug/mL upon 48 hours of exposure, indicating that mercury is highly toxic. A dose-response relationship was recorded with respect to gene induction. Overall, nine out of the thirteen recombinant cell lines tested showed inductions to statistically significant levels ($p<0.05$). At 2.5 ug/mL of mercury, the average fold inductions were 5.2 ± 0.9, 21.4 ± 3.9, 7.0 ± 6.2, 6.8 ± 1.1, 2.7 ± 1.0, 4.5 ± 2.0, 7.5 ± 6.0, 2.2 ± 0.7, and 2.5 ± 0.3, for GSTYa, HMTIIA, *c-fos*, HSP70, CRE, *p*53RE, GADD153, GADD45, and GRP78, respectively. These results indicate the potential of mercury to undergo biotransformation in the liver (GSTYa), and to cause protein damage (HMTIIA, HSP70 and GRP78), cell proliferation (*c-fos*), metabolic perturbation (CRE), growth arrest and DNA damage (GADD153, GADD45), and apoptosis (p53RE). No significant inductions ($p>0.05$) were observed for CYP1A1, XRE, NF*k*BRE, and RARE. (Research supported by NIH-RCMI Grant No. 1G12RR13459).

211 PHOSPHATIDYLINOSITOL 3-KINASE REGULATES ACTIN-MEDIATED NUCLEAR TRANSLOCATION OF NF-E2-RELATED FACTOR 2 (NRF2) FOR RGSTA2 INDUCTION BY *TERT*-BUTYLHYDROQUINONE.

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Expression of phase II detoxifying genes is regulated by Nrf2-mediated antioxidant response element (ARE) activation. We previously showed that phosphatidylinositol 3-kinase (PI3-kinase) plays an essential role in ARE-mediated rGSTA2 induction by oxidative stress. In view of the fact that the signaling pathways of platelet-derived growth factor (PDGF) β receptor tyrosine kinase (RTK) and PI3-kinase control microfilaments and translocation of actin-associated proteins, the current study was designed to investigate the role of these kinases in the rise of $[Ca^{2+}]i$ and actin-mediated translocation of cytoplasmic Nrf2 to nucleus. tert-Butylhydroquinone (t-BHQ) phosphorylated PDGF β receptor, and stimulated a rise in $[Ca^{2+}]i$ and nuclear translocation of Nrf2 in H4IIE cells, which were prevented by pretreatment of the cells with PDGF RTK or PI3-kinase inhibitors (AG1295; wortmannin / LY294002). Chelation of $[Ca^{2+}]i$ suppressed Nrf2 migration and rGSTA2 induction. t-BHQ relocalized Nrf2 in concert with changes in actin microfilament architecture, as visualized by superposition of immunochemically stained Nrf2 and fluorescent phalloidin-stained actin. Furthermore, t-BHQ increased the level of nuclear actin, co-immunoprecipitated with Nrf2, which returned to that of control by pretreatment of the cells with PI3-kinase inhibitors. Cytochalasin B, an actin disruptor, alone stimulated actin-mediated nuclear translocation of Nrf2 and ARE-dependent rGSTA2 induction. The ARE band intensity was decreased by anti-Nrf2 or anti-actin antibody. In contrast, phalloidin, an agent that prevents actin filaments from depolymerization, inhibited Nrf2 translocation and rGSTA2 induction by t-BHQ. Thus, PI3-kinase signaling pathway regulates a rise in $[Ca^{2+}]i$ and rearrangement of actin microfilaments in response to t-BHQ. Also, depolymerization of actin causes actin-bound Nrf2 to translocate into nucleus and stimulates binding of the complex to ARE for rGSTA2 induction.

212 TRANSCRIPTIONAL ACTIVATION OF STRESS GENES, AND CYTOTOXICITY IN HUMAN LIVER CARCINOMA (HEPG2) CELLS EXPOSED TO PENTACHLOROPHENOL.

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Pentachlorophenol (PCP) is a biocidal chemical with several industrial, agricultural, and domestic applications. There is accumulating evidence indicating that PCP is highly toxic to humans, with major target organs including the lung, liver, kidneys, heart, and brain. Evidence of PCP-induced mutagenicity, and carcinogenicity has also been demonstrated in recent studies. However, little is known regarding its molecular mechanisms of toxicity. This research was therefore designed to assess the cellular and molecular responses of $HepG_2$ cells following exposure to PCP. Cytotoxicity was evaluated using the MTT-assay for cell viability, while the CAT-Toxicology (L) assay was performed to measure the transcriptional activation of stress genes in thirteen different recombinant cell lines generated from $HepG_2$ cells. The cyctotoxicity experiment yielded a LD_{50} value of 23.4 ± 5.6 ug PCP /mL upon 48 hrs of exposure, indicating that PCP is acutely toxic. A dose-response relationship was recorded with respect to gene induction. For example, fold inductions of CYP1A1 were 1.0 ± 0.0, 1.0 ± 0.0, 1.3 ± 0.5, 6.3 ± 4.3, and 22.5 ± 3.5 for 0, 6.2, 12.5, 25, and 50 ug PCP /mL, respectively. Overall, five out of the thirteen recombinant cell lines tested showed inductions to statistically significant levels ($p<0.05$). At 50 ug PCP /mL, the average fold inductions were 22.5 ± 3.5, 52.8 ± 2.5, 8.4 ± 1.9, and 12.5 ± 6.8, for CYP1A1, XRE, HMTIIA, *c-fos*, and GADD153, respectively. These results indicate the potential of PCP to undergo Phase I biotransformation in the liver (CYP1A1, XRE), to cause cell proliferation (*c-fos*), growth arrest and DNA damage (GADD153), and to influence the toxicokinetics of metal ions (HMTIIA). Marginal inductions were recorded for HSP70, CRE, RARE, GADD45, and GRP78. Within the dose range (0-100 ug/mL) tested, no significant inductions ($p>0.05$) were observed for GSTYa, NF*k*BRE, and p53RE. (Research supported by Title III Grant No. PO31B990006).

213 UNRESTRICTED BASE PAIRS WITHIN THE ARE/EpRE CORE SEQUENCE OF *c-Ha-ras* CONFER UNIQUE PROTEIN BINDING SPECIFICITY IN VASCULAR SMOOTH MUSCLE CELLS.

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BENZO(A)PYRENE (BaP)-induced oxidative stress activates nuclear protein binding to human *c-Ha-ras* antioxidant/electrophile response element (ARE/EpRE) in vascular smooth muscle cells. The minimal consensus sequence of the ARE/EpRE has been defined as 5'-GTGACNNNGC-3'. Using a 27 base pair (bp) oligonucleotide containing the core *c-Ha-ras* ARE/EpRE sequence in genomic context (hHaras27), two specific DNA:protein complexes of variable inducibility are resolved in electrophoretic mobility shift assays in response to BaP and its metabolites, and shown to be of different protein composition. Flanking sequences to the ARE/EpRE core of *c-Ha-ras* participate in the regulation of nuclear protein complex assembly. The studies presented here were conducted to evaluate the site speci-

ficity of proteins binding to the *c-Ha-ras* ARE/EpRE core. Nuclear protein binding to hHaras27 was absent upon incubation of nuclear extracts with the same oligonucleotide containing a fully mutated ARE/EpRE core region (hHaras27WOUC). Low molecular weight proteins (p23 and p25) previously shown to interact with the ARE/EpRE core of *c-Ha-ras* were not detected upon UV crosslinking of nuclear protein to this mutated sequence, implicating these proteins in core sequence binding. UV crosslinked binding of p23 and p25 to an oligonucleotide comprising only the core *c-Ha-ras* ARE/EpRE sequence (hHaras10) could not be competed by 10bp ARE/EpRE core sequences of human NQO_1 and rat GSTA1 genes, indicating that recognition by these proteins may be unique to the *c-Ha-ras* ARE/EpRE. The only difference in sequence between these ARE/EpRE cores is the "NNN" sequence of the consensus site, suggesting that p23 and p25 bind specifically to the core *c-Ha-ras* ARE/EpRE, and that unrestricted base pairs in the consensus sequence may confer both specificity and uniqueness of protein binding to the ARE/EpRE core. (Supported by NIH grants ES04849 and ES09106 to KSR. KPM is a predoctoral fellow on NIEHS Training Grant ES07273).

214 UPREGULATION OF CYTOKINE RELEASE BY SULFUR MUSTARD EXPOSURE IS MEDIATED BY THE p38 MAP KINASE SIGNALING PATHWAY.

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Sulfur mustard (SM) is a potent alkylating agent that induces skin vesication after cutaneous exposure. Previous work has revealed that SM induces a significant increase in the production of inflammatory cytokines, including IL-8, IL-6, TNF-α, and IL-1β, in cultured keratinocytes and skin. The p38 MAP kinase signaling pathway is activated in response to cellular stress and has been implicated in the upregulation of cytokines in response to inflammatory stimuli. We investigated whether the p38 pathway mediates inflammatory cytokine upregulation in response to SM exposure. A dose-response study in cultured human epidermal keratinocytes (HEK) revealed that p38 is phosphorylated at SM concentrations that are considered to be vesicating doses (≅100 μM) but not at lower doses, which result in DNA damage but not vesication. A time course at the 200 μM exposure revealed that p38 phosphorylation is induced by 15 minutes post-exposure, peaks at 30 minutes and is sustained at peak levels until 8 hours post-exposure. Examination of MKK3/MKK6, which are two closely related kinases that activate p38, revealed a slightly faster rate of phosphorylation of MKK3/MKK6 compared with phosphorylation of p38. Assay of the SM-exposed HEK culture media for cytokines revealed that exposure to 200 μM SM increased IL-8, IL-6, TNF-α, and IL-1β beginning at two hours post-exposure, but had little effect on IL-10 or IL-12(p70). When cells exposed to 200 μM SM were treated with the p38 inhibitor SB203580, the levels of IL-8, IL-6, and TNF-α were decreased ~80% and the levels of IL-1β were decreased ~60% when compared with cells that were not treated with SB203580. These results show that the p38 MAP kinase signaling pathway is an important mediator of SM-induced cytokine production and suggest that directly targeting this signaling pathway with pharmacological inhibitors may alleviate the profound inflammatory response elicited by cutaneous SM exposure.

215 CLONING AND CHARACTERIZATION OF THE ATLANTIC KILLIFISH RETINOBLASTOMA TUMOR SUPPRESSOR cDNA AND INTERACTION WITH THE ARYL HYDROCARBON RECEPTOR.

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The retinoblastoma tumor suppressor gene product (pRB) controls the cell cycle by repressing transcription of genes necessary for S-phase entry, interacting with the p53 apoptosis pathway, and facilitating terminal differentiation. Recent work on mammals has demonstrated that pRB interacts with the aryl hydrocarbon receptor (AHR), a member of the bHLH-PAS family of transcription factors. The AHR mediates the toxic effects of TCDD (2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin) and related compounds, but the endogenous ligand and the physiological role of the AHR have yet to be revealed. The Atlantic killifish *Fundulus heteroclitus* expresses two paralogs of the AHR (AHR1 and AHR2), whereas mammals express only one AHR. It is likely that the pleiotropic functions of the one mammalian AHR are partitioned into the two fish paralogs. We hypothesize that *Fundulus* AHR1 and AHR2 differ in their ability to interact with pRB and regulate the cell cycle. In support of this hypothesis, *Fundulus* AHR1 contains the LXCXE-motif and the glutamine-rich region that interact with mammalian pRB, whereas *Fundulus* AHR2 has neither pRB-binding site. We report here the cDNA cloning of *Fundulus* RB by RT-PCR with degenerate primers and RACE. The *Fundulus* RB cDNA (2739 bp) encodes a 913 amino acid protein with a predicted weight of 103.5 kDa. The *Fundulus* pRB amino acid sequence is 50% and 75% identical to the human and medaka pRB sequences, respectively. The A and B pockets (combined) of *Fundulus* pRB are 67% and 81% identical to human and medaka pRB, respectively, and the putative LXCXE-binding backbone is absolutely conserved among these three proteins. Experiments are underway to test interactions between pRB and the two killifish AHR paralogs. Supported by NIH Grants 1F32ES05935, ES07381 (Superfund), and ES06272, and by a grant from the Donaldson Charitable Trust.

216 RUBRATOXIN B INDUCED THE SECRETION OF CYTOKINES IN CULTURED CELLS.

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Rubratoxin B is a potent hepatotoxic and teratogenic mycotoxin produced by certain *Penicillium* fungi. To date, biochemical, cytological and serological changes have been reported, however, the mechanism of its toxicity remains unclear. In this study, induction of cytokine secretion by rubratoxin B was investigated using cultured cells. Tumor necrosis factor (TNF)-α and interleukin (IL)-8 were secreted from 40 and 80 μg/ml rubratoxin B-treated HL60 cells. In 20 and 40 μg/ml rubratoxin B-treated HL60 cells, monocyte chemotactic protein (MCP)-1 was released. These rubratoxin B-induced cytokines are known to promote liver myelocytic cell infiltration, and activate cytokine-recruited cells. As a result, recruited myelocytic cells are considered to contribute to hepatic injury. Since tyrosine kinases are known to play pivotal roles in signal transduction, we investigated the effects of tyrosine kinase inhibitors genistein and emodin. Genistein reduced the release of all three cytokines from rubratoxin B-treated HL60 cells. Likewise, emodin diminished the secretion of MCP-1. Alternatively, emodin reversed on the secretion of TNF-α, and the release of IL-8 was not affected. Since emodin did not impede rubratoxin B-caused TNF-α and IL-8 secretion in HL60 cells, they appeared to be regulated differently from MCP-1 secretion, suggesting that rubratoxin B exerts its toxicity using two or more signal transduction pathways. Other than HL60 cells, HepG2 cells were investigated. Rubratoxin B induced the secretion of IL-8 in HepG2 cells. However, not like the case of HL60 cells, neither TNF-α nor MCP-1 was detected in rubratoxin B-treated HepG2 cells. These results suggest that HepG2 cells are regulated differently from HL60 cells in the secretion of cytokines.

217 A 13 WEEK ORAL TOXICITY STUDY OF SENNA-MIS IN THE ALBINO RAT WITH AN 8-WEEK RECOVERY PERIOD.

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Senna, a known laxative derived from plants, was administered by oral gavage to Sprague-Dawley (Crl:CD®(SD)BR) rats once daily at dose levels of 0, 100, 300, 750 or 1500 mg/kg/day for up to 13 consecutive weeks followed by an 8-week recovery period for selected animals. Dose and treatment-related clinical signs included abnormal feces (abnormal consistency and color) which were seen to varying degrees from animals from all treated groups. Animals receiving 750 or 1500 mg/kg/day had significantly reduced body weight gain and increased water consumption. Notable changes in electrolytes were seen in both serum (increases in sodium, potassium and chloride) and urine (decreases in sodium and increase in potassium and chloride levels). Darker urine was also seen in these groups. At both the terminal and recovery necropsies, an increase in absolute and relative kidney weights (relative to both body and brain weights) was seen for male and female animals receiving 750 and/or 1500 mg/kg/day. Dark discoloration of the kidneys was observed in animals receiving 300 mg/kg/day or above. Histopathological changes following 13-weeks of treatment were seen in the kidneys of animals receiving 300 mg/kg/day and above (slight to moderate, tubular basophillia and tubular pigment deposits). In addition, for all treated groups, minimal to slight hyperplasia in the colon and rectum and minimal hyperplasia of the squamous epithelium of the stomach were recorded. Following 8 weeks of recovery, with the exception of the brown pigment in the kidney, these pathological findings were not evident. Thus, the biochemical and histopathological changes seen after 13 weeks of treatment with Senna, significantly reversed following 8 weeks of recovery.

218 DECREASED GLUTATHIONE LEVELS AND CELL VIABILITY IN NEURONAL AND GLIAL CELLS EXPOSED TO FUMONISIN B1.

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Fumonisin B1 (FB1) is a toxin produced by the fungus *Fusarium verticillioides* (= *F. moniliforme*). FB1 is commonly present in *Fusarium* infected corn, but it is thought that FB1 also could be present in buildings with moisture and mold problems. FB1

is known to cause for example leukoencephalomalacia in horses, pulmonary oedema in pigs and liver cancer in rats. The aim of this study was to try to clarify the mechanisms whereby FB1 affects neuronal and glial cells. Four different cell lines were used: human SH-SY5Y neuroblastoma cells, human U-118MG glioblastoma cells, mouse GT1-7 hypothalamic cells and rat C6 glioblastoma cells. All cell lines were grown in Dulbecco's Modified Eagle Medium in bottles and exposed to FB1 at concentrations of 0.1, 1, 10, 30 and 100 μM for 0-144 h in 6- or 48-well plates. Changes of intracellular glutathione (GSH) levels, cell viability and caspase-3 activity were measured with fluorescent probes, monochlorobimane, propidium iodide and a fluorescent caspase-3 substrate, respectively. Decreased GSH levels were observed in all cell lines after exposure to 100 μM FB1 for 144 h. In U-118MG, GT1-7 and C6 cells decreased GSH levels were also seen at earlier time points. Cell viability of all cell lines exposed to FB1 (1-100 μM) for 144 h was lower than controls. The viability of cells was not affected at earlier time points. Increased caspase-3 levels were observed in GT1-7 cells exposed to 10, 30 and 100 μM FB1 for 72 h. No caspase-3 activation was seen in the other cell lines. The decreased levels of GSH in all cell lines exposed to FB1 for 144 h indicate that long term exposure to FB1 may harm neuronal and glial cells, and the lowered cell viability at this same time point confirms this observation. The activation of caspase-3 in GT1-7 cells suggests that cell death may occur by apoptosis in this cell line. Supported by the Academy of Finland, Finnish Research Programme on Environmental Health.

219 ANALYTICAL METHOD VALIDATION OF KAVA KAVA POWDERED EXTRACT DOSED IN CORN OIL.

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Kava kava powdered extract (kava) is an herbal supplement that has been selected for toxicological evaluation by the NIEHS. A method was validated for the analysis of kava in corn oil formulations. Neat kava and corn oil dosed with kava were extracted under ambient conditions and the extracts were analyzed using an isocratic HPLC method with UV detection at 220 nm. A method validation was performed over a dose range of ∽ 5 to ∽ 500 mg kava/mL corn oil. Solvent standards containing between ∽ 5 and ∽ 500 mg of kava were extracted by sonicating for 60 minutes at ambient temperature with 50 mL of methanol, corresponding to an analytical concentration of ∽ 0.1 to ∽ 10 mg kava/mL methanol. Spiked corn oil standards containing ∽ 5 mg kava/mL corn oil and ∽ 500 mg kava/mL corn oil (analytical concentration of ∽ 0.1 to ∽ 10 mg kava/mL methanol) were extracted in the same manner. All samples were diluted to a final analytical concentration of ∽ 0.1 mg kava/mL methanol. Kavain, one of the major kavalactones, was used to prepare quantitation standards (∽ 3 to ∽ 393 μg/mL) to determine the weight percentages of kavain in kava solvent and spiked corn oil standards. The kavain quantitation standards proved to be linear ($r \geq 0.9999$) and accurate (%RE ≤ 2%). The extraction of kavain from kava solvent and spiked corn oil standards proved to be linear ($r \geq 0.9999$) and precise (%RSD ≤ 1.7%). The average % kavain extracted from the kava in the solvent standards was 6.61 ± 0.11 (s)% (n=10). The average % kavain extracted from the kava in the spiked corn oil standards was 6.65 ± 0.10 (s)% (n=10). The average extraction recovery for kavain from corn oil dosed with kava was 100.8 ± 1.5(s)%.

220 COMPARATIVE METABOLISM OF BREVETOXINS BY RAT AND HUMAN LIVER MICROSOMES.

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Karenia brevis produces a series of neurotoxic cyclic polyethers known as brevetoxins (PbTxs). Recent evidence suggests that the respiratory tract, hemopoietic, and immune systems are potential targets for PbTx toxicity following repeated exposure. We have investigated the comparative cytochrome P450-mediated metabolism of PbTx2 and PbTx3 by Sprague-Dawley (SD) rat, Fischer rat, and human liver (pool of 15 males) microsomes. Brevetoxin 2 (0.25 to 10 μg/ml incubation mixture) was incubated (37°C) with microsomes from each strain and species under conditions of linearity with respect to protein concentration and time. Control incubations were conducted with inactivated microsomes. Extent of metabolism was quantitated by determining the loss of PbTx m+1/z ion in the incubation mixture extracts by LC-mass spectrometry (LCMS). The molecular weights of possible metabolites were also determined by LCMS. The affinity of the cytochrome P450s for PbTx2 (K_m) and maximum velocity of the metabolic breakdown (V_{max}) were calculated. The V_{max}s (mean ± sem; n=3), for PbTx2 metabolism by SD rat, Fischer rat, and humans were 1.72 ± 0.42, 2.02 ± 0.47, and 1.15 ± 0.03 nmole/mg/min, respectively. The K_ms for SD rat, Fischer rat, and human microsomes were 3.80 ± 0.62, 4.98 ± 1.52, and 4.40 ± 0.55 μM, respectively. Potential metabolites, with (m+1)/z = 759, 783, 807, and 832 were found for both species. Brevetoxin 3 did not appear to undergo metabolism. Results to date suggest that rates of cytochrome P450-mediated metabolism of PbTx 2 by two strains of rats and humans are similar on a mg protein basis. Further work is needed to identify and characterize the toxicities of the metabolites. Research funded under NIH P01-ES10594ZES.

221 FLORIDA'S RED TIDE *DINOFLAGELLATE KARENIA BREVIS* MAY MODULATE ITS POTENCY BY PRODUCING A NON-TOXIC COMPETITIVE ANTAGONIST.

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Florida red tides are the result of blooms of the unarmoured *dinoflagellate Karenia brevis*. K. brevis produces 9 different highly potent polyether brevetoxins (PbTx) that bind with high affinity to site 5 of the VSSC. The relative profile of toxins produced by K. brevis depends on growth phase and strain. These two findings may explain the variable potency of individual red tide blooms. A naturally-produced competitive antagonist for brevetoxins is described. The antagonist is found at low concentrations in rapidly-growing K. brevis cells but increases to 20% of the total toxin biomass in senescent cells. The antagonist has low potency in fish bioassay (1ug/mL (n=10) and 2 ug/ml (n=5) produced no 24 hr deaths), and in 1ug/mL combination with PbTx-2 prolonged time until death by 2.5-fold. The antagonist cross-reacts in PbTx-ELISA, indicating an intact H-K ring. Low resolution ESI gave an M + 1 peak, m/z of 657.5. High resolution FAB mass spectroscopy gave a molecular ion, m/z 656.90. Proton NMR of the antagonist indicates that the C-2, C-27 and C-28 hydrogens are no longer unsaturated, and there appears to be additional sites of unsaturation within the molecule. The exomethylene hydrogen unsaturation is also missing, but there appears to be an endomethylene in conjugation with the terminal aldehyde. The structure of the antagonist appears to be similar to the hemibrevetoxins in that it has an abbreviated PbTx-2 backbone composed of at least the H-K rings and most likely include the E-K or F-K rings with further points of unsaturation on side chains. The A-ring activity region is absent. This is the first report of a competitive non-toxic ligand produced by K. brevis, and may explain the rapidly decreasing potency of red tides during their terminal phases in natural environments. It also represents an additional antagonist for nerve membrane study of marine toxins, and joins synthetic antagonists alpha- and beta-naphthoyl brevetoxins.

222 SILYMARIN DOSE-DEPENDENTLY MODULATES IMMUNE FUNCTION IN BALB/c MICE.

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Silymarin, a mixture of bioactive flavanoids isolated from Milk Thistle (*Silybum marianum*), has been used for centuries as a natural remedy and is now effectively used in the treatment of liver toxicity and disease in humans. *in vitro* studies show that silymarin can inhibit the production and damage caused by tumor necrosis factor α (TNFα). It is also a potent antioxidant both *in vitro* and *in vivo*. These studies suggest silymarin may impact the immune system but little information exists following *in vivo* exposure. Therefore, we tested the hypothesis that exposure to silymarin will modulate the inflammatory immune response. Male BALB/c mice (6/group) were treated intraperitoneally once daily for five days with 0, 10, 50 or 250 mg/kg of silymarin. Splenic cultures were stimulated with 1 μg/ml of LPS for 6 hrs following which gene expression was analyzed by reverse transcriptase-polymerase chain reaction. Silymarin exposure did not produce any signs of overt toxicity or any changes in relative organ weights. Functional examination of secondary lymphoid tissue revealed that phytohemagglutinin-induced T-lymphocyte proliferation was increased in the lowest dose group only. B-lymphocyte blastogenesis induced by lipopolysaccharide was increased following exposure to 10 and 50 mg/kg of silymarin. Flow cytometric examination of splenic lymphocyte populations showed that the absolute number of $CD3^+$ T-lymphocytes was reduced in the 10 mg/kg group only. A concomitant decrease in the $CD4^+$ T-helper cell population was evident in this group. Similarly, the expression of interleukin (IL)-2 and IL-4 were decreased in the 10 mg/kg group only. In contrast, expression of TNFα, inducible nitric oxide synthase, IL-1β and IL-6 mRNA were increased dose-dependently. The results indicate that *in vivo* exposure to silymarin results in suppression of T-lymphocyte function at low doses and stimulation of inflammatory processes at higher doses. Further studies investigating the effects of silymarin on the immune system are warranted. (Supported in part by NIH TW01009)

223 EFFECTS OF SILYMARIN ON REGIONAL BRAIN NEUROTRANSMITTER LEVELS IN BALB/c MICE.

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Natural antioxidant silymarin is a recognized remedy widely used in the treatment of liver pathologies. The effect of this flavonoid mixture upon the central nervous system is unknown. Brain amine metabolism was studied after silymarin treatment. BALB/c mice were intraperitoneally administered 0, 10, 50 or 250 mg/kg of silymarin per day in the course of 5 days. Mice were sacrificed 24 hours following the final treatment and brains were dissected into seven regions: cerebellum, medulla oblongata, hypothalamus, striatum, thalamus, hippocampus and cortex. There were no differences in brain region weights between treatment groups. High performance liquid chromatography coupled with electrochemical detection was performed to determine concentrations of norepinephrine (NE), dopamine (DA), dioxyphenylacetic acid, homovanilic acid, 5-hydroxytryptamine (5-HT) and hydroxyindoleacetic acid in discrete regions. Analysis showed changes of brain amines levels in two brain regions, the cortex and cerebellum, while no alterations in remaining regions were evident. Exposure to silymarin increased 5-HT level in cortex in a dose-dependent manner. In cerebellum elevated levels of DA and NE were detected; however, increases were only noted in animals exposed to the highest concentration (250 mg/kg) of silymarin compared to the control group. No response was observed in the striatum and hypothalamus, regions known for highest NE and DA activity. Results indicated that the overall effects of silymarin treatment upon brain amine metabolism are minor. The increases observed at the highest dose level, although significant, remained within physiological levels. It may be concluded, that silymarin is safe regarding brain amine homeostasis up to the doses employed under the present experimental conditions. (Supported in part by NIH TW01009)

224 SILYMARIN ALTERS THE EXPRESSION OF CYTOKINES IN MOUSE LIVER.

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Silymarin is a flavanoid mixture extracted from *Silybum marianum* (St. Mary's thistle), and its main components consist of silibinin, isosilibinin, silicristin, and silidianin. It has been shown to be protective in various forms of liver diseases including alcohol- and drug-induced problems, and acute and chronic hepatitis. Silymarin may be hepatoprotective due to its antioxidant activity, increase in cellular glutathion content, membrane stabilizing effect, and increase of protein synthesis by RNA polymerase activation. The present study investigated the effect of silymarin on expressions of hepatic cytokines and factors involved in inflammatory reactions and cell growth. BALB/c mice (6-7 weeks old) were randomly divided into 4 groups of 6 mice each and treated with 5 daily intraperitoneal injections of silymarin at 0, 10, 50, or 250 mg/kg body weight. Cytokine gene expression was analyzed by reverse transcriptase-polymerase chain reaction. Silymarin treatment resulted in dose-dependent but insignificant decreases in plasma alanine aminotransferase and aspartate aminotransferase. Silymarin caused dose-dependent increases of the transforming growth factor β1 (TGFβ1) and c-*myc* expression in liver; significant differences were observed at 250 mg/kg group. Dose-related insignificant increases were noted in the expression of hepatocyte growth factor and interferon-γ. Silymarin treatment did not change the expression of tumor necrosis factor-α or class II major compatibility complex in the liver. It has been reported that TGFβ1 is fibrogenic in liver, and c-*myc* can induce cell proliferation or apoptosis under different conditions. Results suggest that long-term, high dose administration of silyamrin may prevent or induce hepatic damage due to a persistent disruption of TGFβ1 and c-*myc* expression. (Supported in part by NIH TW01009).

225 ASSESSMENT OF CYTOTOXICITY OF INDIGENOUS COSMETIC INGREDIENTS IN VERO CELLS.

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There is an increase in the application of natural products in cosmeceutical industries worldwide. In an attempt to assess the cytotoxicity of the local Malaysian plant products, an *in vitro* cytotoxicity study was carried out using a mammalian Vero monkey kidney cells. The uptake of the vital dye neutral red (NR) into the lysosomes/endosomes and vacuoles of living cells were used as a quantitative indication of cell number and viability. Indigenous cosmetic ingredients of two essential oils of Citronella (Cymbopogon nardus, also known as serai wangi) and Tea tree (Melalleuca sp.), and a commercially available natural surfactant namely Plantacare 1200 were assessed using this method. The IC_{50} value, defined as the concentration of compound that caused a 50 % reduction in the uptake of NR when compared to the controls was determined. Our results show that Citronella oil's IC_{50} value is 108.6 ± 13.2 µg/ml which is comparable to tea tree oil's IC_{50} of 113.7 ± 6.3 µg/ml. The IC_{50} of Plantacare 1200 is 79.6 ± 4.5 µg/ml, which is relatively more toxic than our two indigenous plant ingredients. In conclusion, we have demonstrated that the cytotoxicity of Citronella and Tea tree oil extracted from our local sources are relatively safe as compared to the commercially available surfactant which is used in the formulation of the cosmetics. (This study was funded by IRPA grant no. 09-01-01-0021)

226 INHIBITION OF INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSION BY SILYMARIN IN LPS-STIMULATED MACROPHAGES.

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Silymarin, a polyphenolic flavonoid antioxidant, has been shown to have anti-inflammatory, hepatoprotective, and anticarcinogenic effects. In the present study, we report the inhibitory effect of silymarin on nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) mRNA expression in macrophages. *in vivo* administration of silymarin attenuated NO production of peritoneal macrophages in lipopolysaccharide (LPS)-treated mice. Silymarin also produced dose-dependent suppression of LPS-stimulated production of NO in isolated mouse peritoneal macrophages and macrophage cell line RAW264.7. Moreover, treatment of RAW264.7 with silymarin inhibitied LPS-stimulated expression of iNOS mRNA in a dose-related manner. To further investigate the mechanism responsible for reduced iNOS gene expression, we investigated the effect of silymarin on LPS-stimulated activation of NF-κB/REL, which regulates various genes involved in immune and inflammatory response. LPS-stimulated DNA binding of NF-κB/REL was inhibited by silymarin, and this effect was mediated through inhibition of phosphorylation and degradation of IκBα. NF-κB/REL-dependent reporter gene expression was also suppressed by silymarin. Collectively, these data suggest that silymarin may inhibit NO production and iNOS mRNA expression *via* inhibition of NF-κB/REL activation.

227 PBPK MODEL FOR UPREGULATION OF THE THYROID DUE TO PROLONGED PERCHLORATE EXPOSURE.

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A preliminary model was developed to account for upregulation of thyroidal iodide (I^-) uptake after subchronic perchlorate (ClO_4^-) exposure. A previous physiologically based pharmacokinetic (PBPK) model was capable of predicting ClO_4^- induced inhibition of I^- uptake at the sodium iodide symporter (NIS) after acute perchlorate exposure, but did not account for upregulation by thyroid-stimulating hormone (TSH). Naive thyroid follicular Vmax values (approximately1.5 and 0.7 mg/hr/g tissue for I^- and ClO_4^-, respectively) were estimated by visually fitting radioiodide uptake data at baseline TSH levels (prior to ClO_4^-). TSH upregulates the thyroid by stimulating an increase in NIS synthesis, thereby increasing iodide transfer into the thyroid. In this model the increase in NIS as a result of prolonged perchlorate exposure is estimated by adjusting the thyroid follicular Vmax. A mathematical equation was derived that is capable of expressing changes in Vmax, which are dependent upon circulating serum ClO_4^- levels and perchlorate's affinity to NIS. In addition, the "upregulated" Vmax is expressed as a time dependant function. Utilizing this method, the model predicts the time and dose dependant behavior of I^- uptake in the rat thyroid during 14 days of ClO_4^- exposure.

228 TOWARDS A BBDR MODEL FOR 5-FLUOROURACIL EMBRYOTOXICITY: ADDING A PBPK MODEL AND A SUBMODEL FOR THE TIME DELAY IN THYMIDYLATE SYNTHETASE INHIBITION.

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5-Fluorouracil (5-FU) is a widely used chemotherapeutic drug for the treatment of solid tumors, and is a known teratogen in a variety of test species. 5-FU has an extensive literature and was chosen as a prototypic toxicant to construct a biologically

based dose-response (BBDR) model for developmental toxicity (Shuey et al., 1994). Setzer et al. (2001) developed the BBDR model using a mechanistic framework for 5-FU's embryotoxicity and dose-response data provided by Lau et al. (2001). The proposed mode of action is as follows: 1. distribution of parent 5-FU to the fetus -> 2. anabolism to fluorodeoxyuridylate (FdUMP) -> 3. FdUMP inhibition of the DNA pyrimidine forming enzyme, thymidylate synthetase (TS) -> 4. depletion of the TS product, thymidine triphosphate (dTTP), and nucleotide pool perturbation -> 5. altered cell cycle and cell replication -> 6. fetal weight deficits, cell death, and dysmorphogenesis. The initial BBDR model consisted of semi-empirical submodels for steps 1-4, and parameter values derived from the dose-response data. Simulation results adequately explained the TS inhibition response, but an unexplained time delay between peak 5-FU levels and peak TS inhibition was modeled empirically. To incorporate more of the underlying physiology, the original three compartment kinetic model was replaced with a multicompartment pregnant rat PBPK model (O'Flaherty et al., 1991) that simulated the distribution of 5-FU based upon its pKa and the pH of tissues, and that accounted for growth in the maternal tissues and fetus throughout the pregnancy. The empirical model for the TS inhibition time delay was replaced with a model of the kinetics for the FdUMP-TS-folate complex formation and competition from the naturally occurring TS substrate, deoxyuridylate (dUMP). These more physiologically realistic models improved the simulation of the TS inhibition dose-response, and the utility of the model to predict effects from different dosage regimens or in different species including humans. [This abstract does not necessarily reflect EPA policy.]

229 PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL PARAMETER ESTIMATION AND SENSITIVITY AND VARIABILITY ANALYSES FOR ACRYLONITRILE DISPOSITION IN HUMANS.

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A physiologically based pharmacokinetic model of acrylonitrile (ACN) and cyanoethylene oxide (CEO) disposition in rats was developed (Kedderis et al., 1996) and extended to humans (Kedderis, 1997; Kedderis and Held, 1998). This work further refines previous models by refining the approach to scaling metabolic parameters from rat to human using *in vitro* data, incorporating an *in vitro-in vivo* correction factor (calculated from the rat) and reflecting species differences in liver size, enzyme content, co-factor (glutathione) concentrations, and body weight. Estimated human biotransformation rates *in vivo* were generally increased 3- to 7-fold from previous estimates. For a given exposure concentration, refined model predictions of rat and human tissue or blood CEO concentrations were generally found to be similar. Sensitivity and variability analyses were also conducted on the refined model. While many parameters contribute to the estimated variability of the model predictions, reaction rate of CEO with glutathione, hydrolysis rate for CEO, and blood:brain partition coefficient of CEO were the parameters that made the greatest contributions to variability. Improved estimates of these parameter values and their variability present an opportunity to improve the understanding of human blood and tissue dosimetry following acrylonitrile exposure.

230 A PHYSIOLOGICAL PHARMACOKINETIC MODEL BASED ON TISSUE LIPID CONTENT FOR SIMULATING INHALATION PHARMACOKINETICS OF HIGHLY LIPOPHILIC VOLATILE ORGANIC CHEMICALS.

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The highly lipophilic volatile organic chemicals (HLVOCs) are distributed almost uniquely in the neutral lipid fraction of tissues and blood. As suggested by their high *n*-octanol:water partition coefficient (>8000), their solubility in water fraction of tissues and blood is negligible. Hypothetically then the kinetics of HLVOCs can be simulated solely with the consideration of their solubility and distribution in neutral lipid-equivalent (NLE) fractions of the tissues and blood. The objectives of the present study were therefore: (i) to develop a physiological pharmacokinetic model based on NLE content of tissues and blood, and (ii) to apply this model framework for simulating the inhalation pharmacokinetics of HLVOCs (i.e., *d*-limonene, α-pinene, 1, 2, 4-trimethylbenzene and *n*-hexane) in rats and/or humans. The PBPK model developed in this study consisted of tissue compartments that represented only their NLE content. All biological parameters, except alveolar ventilation rate, were expressed on the basis of their NLE content. Tissue:blood partition coefficients were not used since the solubility of HLVOCs in tissue neutral lipids and blood neutral lipids is considered to be the same. The NLE-based physiological pharmacokinetic model developed in this study described adequately the uptake and disposition kinetics of α-pinene, *d*-limonene and 1, 2, 4-trimethylbenzene in humans and that of *n*-hexane in rats. The NLE-based model framework developed in this study represents an original, mechanistic tool for simulating the lipid concentrations and pharmacokinetics of HLVOCs without the use of tissue:blood partition coefficients.

231 PREDICTABILITY OF THE PERMEATION-AREA CROSS PRODUCT FOR SIMULATING TISSUE UPTAKE OF CHEMICALS IN PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS.

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In PBPK models, the tissue uptake of non-ionized organic chemicals (NIOCs) is described as a perfusion-limited process. Conceptually, this process reflects passive diffusion of chemicals across capillary membranes according to Fick's law. For calculating chemical flux as per Fick's first law, diffusion coefficient (D), membrane thickness (T), and area of the membrane (A) should be known. In physiological models, chemical flux is calculated with the knowledge of permeation-area cross product, i.e., PA(=DxA/T) as well as tissue blood flow rate (Qt) according to the equation: PAxQt/(PA+Qt). When the PA value is very large compared to Qt, the latter (i.e., Qt) is used as the determinant of chemical flux between tissues and blood. Actually, PBPK models for NIOCs use Qt as the determinant of chemical flux without any knowledge of PA. The objective of the present study was to develop an approach for predicting PA for various tissues of human PBPK models, and to compare them with Qt, using styrene as the example. For computing the values of PA of chemicals, the D values were obtained using the relationship, log D=-3.20234 logMolWt+1.974, whereas the T for capillaries was set to 0.001 cm and the values of A (x1000 cm^2) for capillaries in various tissues were obtained following a review of the literature (liver: 98, adipose tissues 200, slowly perfused tissues: 402, richly perfused tissues: 300). The results of this study suggest that, for chemicals with PA values greater than 230, 310, 980 and 1100 L/hr, Qt is the limiting factor of tissue uptake in the adipose tissue, slowly perfused tissues, liver and richly perfused tissues. Further, using the tissue-specific PA values calculated for styrene, the present study showed that the perfusion-limited PBPK model is in fact appropriate for this chemical. This study represents the first attempt to predict PA values and provides the basis for determining the appropriateness of the use of perfusion-limited description of tissue uptake of chemicals in PBPK models.

232 MAGNITUDE OF INTERSPECIES DIFFERENCES IN TISSUE:AIR PARTITION COEFFICIENTS USED IN PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS OF VOLATILE ORGANIC CHEMICALS.

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Tissue:air partition coefficient (P_{ta}) corresponds to the ratio of solubility or equilibrium concentration of chemicals in two matrices. A common default approach in PBPK modeling is to assume that P_{ta} is species-invariant. Neither the validity of this assumption nor the magnitude of the interspecies differences in P_{ta} is known. The objective of the present study was to estimate the magnitude of interspecies differences (rat vs human; mouse vs human) in tissue:air partition coefficients (PCs) of organic chemicals. The approach involved the calculation of the species-specific P_{ta} for several chemicals with n-octanol:water PC (P_{ow}) values ranging from 0.01 to 10000, and dividing the human P_{ta} with the animal P_{ta}. The computation of chemical-specific P_{ta} required data on lipid and water contents. These data for the various rat, mouse and human tissues were obtained from the literature. The results of this study suggest that the maximal value of human/rat ratio is about 3 for muscle:air PCs whereas this ratio is near unity for adipose tissue:air and liver:air PCs. The calculated maximal value of human/mouse ratios for muscle:air and liver:air PCs are 1.4 and 0.4, respectively. These results suggest that adipose tissue:air and liver:air PCs determined using rat tissues can be used directly in human PBPK models whereas the muscle:air PC should preferably be determined using human tissues. Alternatively, if the P_{ow} of a chemical is known, the factors developed in the present study can be used to estimate the animal-human adjustment factors applicable for the tissue:air PCs. The present study for the first time has estimated the magnitude of rodent-human differences in tissue:air partition coefficients of organic chemicals based on tissue composition data.

233 AN APPROACH FOR ESTIMATING THE HEPATIC EXTRACTION RATIO OF VOLATILE ORGANIC CHEMICALS (VOCS) FOR HUMAN PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS.

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The metabolism rate constants (V_{max}: maximal velocity for metabolism; K_m: Michaelis affinity constant) or alternatively the hepatic extraction ratio ($E = CL_{int}/[Ql + CL_{int}]$ where $CL_{int} = V_{max}/K_m$ and Ql = liver blood flow rate) for PBPK

modeling are obtained from *in vitro* or *in vivo* studies. Validated *in silico* approaches for predicting V_{max}, K_m or E are yet to become available. The objective of this study was to develop an approach for estimating the hepatic extraction ratio (E) using the steady-state blood concentration (Cb_{ss}) of chemicals in humans. The approach consisted using experimental values of Cb_{ss} of toluene (T), *m*-xylene (X) and ethylbenzene (E) in order to estimate their apparent blood:air partition coefficient (P_{ba}). Knowing the fraction of blood flow to the liver (Ql_c) and the blood:air partition coefficient (P_b) of the chemical, it was possible to estimate E (0.75 T; 0.85 X; 0.73 E) according to the following algorithm: $P_{ba} = P_b/(1+Ql_cEP_b)$ derived from the PBPK model. The relationship between the P_{ba} and the P_b of several (19) volatile organic chemicals was also investigated. A log-linear relationship ($\log P_{ba} = 0.6897 \log P_b - 0.1167$) was found to adequately reflect the quantitative relationship between P_{ba} and P_b, and this relationship indicates the feasibility of predicting E from knowledge of P_b of VOCs. The results of this study demonstrate the adequacy of using blood concentration data and P_{ba} for estimating E for human PBPK models of VOCs.

234 A SPECIALIZED COMPARTMENTAL MODEL FOR THE DISPOSITION OF FAT-ACCUMULATING XENOBIOTICS IN ADIPOSE TISSUE: APPLICATION TO A SYSTEMIC PBPK MODEL FOR TRICHLOROETHYLENE (TCE).

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Large numbers of xenobiotics are known to accumulate in fat tissue, including many organochloride pesticides, PCBs, dioxins and volatile organic compounds. This accumulation in the fat has a major effect on the overall pharmacokinetics of such compounds, including blood and target tissue concentrations. The purpose of this research was to develop a PBPK model for the disposition of the fat-accumulating solvent TCE, with a focus on better describing fat concentrations. Preliminary efforts indicated that the standard PBPK models were not able to capture the dynamics of TCE concentrations in the fat tissue of adult male Sprague-Dawley rats exposed to 50 ppm TCE *via* inhalation for up to 2 hours (unpublished data). Moreover, the assumptions of the standard PBPK models were inconsistent with variations in fat cell size, lipid distribution, and blood flow within a fat depot. These physiological characteristics and the preliminary modeling results led to the development of a specialized compartmental model for TCE in fat tissue based specifically on the physiology. This spatially-varying model included capillary, interstitial fluid and fat cell subcompartments that were based directly on the structure of fat tissue. A dispersion-type term was used to account for the heterogeneities in the fat, and the resulting model was coupled with standard compartments for the blood and remaining tissues to create a systemic PBPK model for TCE. Parameter values were from a PBPK model for TCE (MV Evans, unpublished results) or were based on known physiological properties. Model simulations suggested that the specialized fat compartmental model was best able to capture the dynamics of TCE within the fat. In summary, a dispersion-type fat model is a promising tool in PBPK modeling for accurately describing the disposition of fat-accumulating compounds. (This abstract does not reflect EPA policy).

235 THE IMPORTANCE OF STRAIN-SPECIFIC METABOLIC PARAMETERS IN PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS: CARBON TETRACHLORIDE AS AN EXAMPLE.

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The goal was to determine effects of physiological parameters on our ability to estimate metabolic parameters for a model volatile organic compound (VOC), such as carbon tetrachloride (CCl4). Using a published PBPK model for CCl4, we estimated maximum metabolic rate (Vmax) and affinity of CCl4 for the metabolic enzyme (Km) for two rat strains, Sprague-Dawley (S-D) and Fischer 344. Of nine strain-specific physiological parameters, five parameters were changed, since four were approximately identical. The five parameters unique for each strain were: alveolar ventilation rate, cardiac output, percent muscle volume, percent blood flow to liver, and percent blood flow to muscle. We performed optimizations against the same set of experimental data to evaluate the difference in Vmax and Km estimates due to the combined influence of these parameters. The resulting estimates for Vmax and Km were only slightly different. Once these metabolic estimates were determined, we repeated the simulations with the new Vmax and Km estimates and compared chamber concentration (Cp) and liver concentration (Cl) for each rat strain. Simulations incorporating strain-specific physiological and updated metabolic parameters produced a difference in both model outputs. The percent difference (Fischer Cl minus S-D Cl divided by S-D Cl) was larger for liver concentration compared to chamber concentration at all initial concentrations. At the lowest simulated concentration (25 ppm), the maximum percent difference was 32% for liver concentration compared to 10% for chamber concentration. At the highest simulated concentration (1000 ppm), the maximum percent difference was 50% for liver concentration compared to 7% for chamber concentration. Since accurate metabolic parameter estimates for VOCs are necessary for dose-response assessment, this research indicates the importance of including strain-specific values in PBPK models for similar chemical compounds. (This abstract does not necessarily reflect EPA policy.)

236 CORRELATION OF HEPATIC DAMAGE AND TARGET DOSE USING A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR CHLOROFORM AND BROMODICHLOROMETHANE.

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Chloroform (CHCl3) and bromodichloromethane (BDCM) are members of the trihalomethane family of disinfection by-products resulting from chlorination of source water. The application of PBPK modeling has been long proposed to estimate target dose for specific chemicals. The purpose of this research was to use PBPK models for both chemicals to estimate measures of liver dose and to correlate these results with serum liver damage markers following an oral gavage exposure in aqueous media. Oral gavage was conducted using Fischer 344 male rats approximately 90 days old. Two published data sets were combined to include the following dose range: 0, 0.125, 0.19, 0.25, 0.5, 0.75, 1, 1.5, 2, and 3 mmoles/kg. Serum alanine aminotransferase (ALT), a marker of hepatic damage, was measured 24 hours after exposure. Physiological and age specific values for Fischer 344 male rats were used with previously published PBPK models for both chemicals. The models were structurally similar except for differences in partition coefficients and metabolic parameters. The correlation of several predicted dose metrics with serum ALT was evaluated. The model predictions used for comparison were: peak rate of amount metabolized, total amount metabolized, and peak liver concentration of the parent chemical. Two of these dose metrics correlated well with ALT for both chemicals: total amount metabolized and peak liver concentration. However, it is currently believed that the hepatic toxicity of CHCl3 and BDCM require metabolic activation in order to induce damage. The model simulations presented here, coupled with metabolic activation, and recently published mechanistic data for CHCl3, all suggest that total amount metabolized is a useful metric when describing hepatic damage. In summary, PBPK modeling was shown to be a useful tool for suggesting potential mechanisms of action for two related volatile chemicals. (This abstract does not reflect EPA policy).

237 DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR ARSENIC.

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Risk assessment for arsenic is uniquely complex due to both the variety of toxicologically active forms and uncertainty concerning their relative roles in the causation of arsenic-induced cancer and non-cancer health effects. We have developed a PBPK model for arsenic to provide an integrated framework for hypothesis testing and experimental design with the ultimate goal of linking this model to the biological cascade of events leading to arsenic-induced health effects. The model development strategy is based on use of data from our pharmacokinetic studies in mice with each of the major stable forms of arsenic - arsenate (AsV), arsenite (AsIII), monomethylarsonic acid MMA(V) and dimethylarsinic acid DMA(V). The model development strategy has involved sequential separate development of each of four submodels working backwards from dimethylarsenic (DMA) thru monomethylarsenic (MMA), AsIII and AsV. The models are linked by reduction and subsequent methylation steps with urinary and fecal excretion in each of the submodels. All of the submodels include the following compartments representing relevant organs and tissues: arterial and venous blood, lung, liver, kidney, bladder, and residual body. Estimates for partition coefficients were derived from previously published studies using numerical methods. In general distribution into tissues was modeled assuming blood flow limited kinetics in plasma. The notable exception to this was the inclusion of a "deep lung" compartment in the DMA submodel to account for prolonged retention of DMA in lung whether given as parent compound or as AsV or AsIII. Good visual fits were obtained for much of the data used to evaluate the model; however, liver was often a notable exception. Sensitivity analysis suggests that binding and methylation parameters are particularly influential in this regard. Our modeling efforts point to the need for additional species-specific *in vitro* studies to provide a robust mechanistic basis for cross-species extrapolation. (This abstract does not necessarily reflect EPA policy).

238 ESTIMATING PREVIOUS SERUM TCDD CONCENTRATIONS IN AN OCCUPATIONALLY EXPOSED COHORT USING A PBPK MODEL.

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This study estimated previous serum concentrations of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) in 134 male workers involved in producing sodium trichlorophenol or a derivative. These workers conceived children either during or after this employment. The estimated TCDD concentrations at the time of conception of children were used in a male reproductive health study (in press). Serum TCDD concentrations were estimated with a PBPK model. Available data were serum TCDD level at the end of the study, dates of employment in dioxin-related processes, and body mass index (BMI) at the end of the study and an earlier time point. A linear regression model estimated missing BMI values for 23 workers who had only one measured BMI. Each worker was assumed to have constant "background" exposure to TCDD, estimated from measured serum TCDD values in 79 controls. Individual occupational TCDD exposure rates were estimated for each worker based on final measured serum TCDD concentration and dates of exposure in TCDD-related processes. TCDD elimination in the PBPK model is proportional to hepatic TCDD concentration; the major site of storage is the fat compartment. The hepatic elimination rate constant for TCDD in this model was previously estimated from the Air Force Ranch Hand study. Although the instantaneous elimination rate of TCDD in the model is first-order, changes in body fat composition over time lead to non-linear kinetics. Unlike other simple first-order models, the volume of distribution of TCDD varies as BMI changes over time. The major conclusions are: (1) the retrospective serum TCDD concentrations are highly dependent on changes in body fat; and (2) data on individual body weight changes, as well as on TCDD exposures, should be gathered when modeling the long-term pharmacokinetic behavior of TCDD in humans. Use of this BMI-linked PBPK model for TCDD permits the investigator to distinguish between plasma kinetics and the whole-body kinetics of TCDD.

239 TISSUE DOSIMETRY BASED ANALYSIS OF SPECIES-SPECIFIC DIFFERENCES IN PULMONARY TOXICITY FROM STYRENE EXPOSURE: A PBPK MODEL-BASED APPROACH.

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Chronic rodent studies demonstrate species specificity in the pulmonary carcinogenic response to inhaled styrene (ST). Increased incidences of bronchioloalveolar tumors was observed in mice, but not in rats. Although the data are not definitive with regard to a target cell, ST-induced lung tumors in mice could be from Clara cells in the respiratory epithelium lining the terminal bronchiole. Clara cells metabolize ST to styrene-7, 8-oxide (SO), which is cytotoxic and weakly genotoxic. Rodent species show marked differences in density and distribution of Clara cells and their metabolic capacity. A physiologically based pharmacokinetic (PBPK) model was developed to predict the concentration of ST and SO in the respiratory tract tissues. The model incorporated a multi-compartment description of the respiratory tract, including species specific quantitative information on respiratory tract physiology and metabolic capacity. The model was validated against multiple data sets. The PBPK model predicts a 10 fold lower SO concentration in the terminal bronchioles in rats compared to mice, which is consistent with the observed species sensitivity to the development of respiratory tract neoplastic lesions. Furthermore, model-estimated SO concentration in the terminal bronchioles of mice show a close correlation to the incidence of neoplastic lesions. However, blood SO concentration do not correlate to the neoplastic lesions data, indicating that the carcinogenic response in rodent lung is controlled by local metabolism rather than systemic delivery.

240 ROUTE-SPECIFIC DIFFERENCES IN DISTRIBUTION CHARACTERISTICS OF OCTAMETHYLCYCLOTETRASILOXANE IN RATS: A PHYSIOLGICAL MODEL BASED ANALYSIS.

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Octamethylcyclotetrasiloxane (D4) has unusual pharmacokinetics resulting from its unique combination of low blood:air and high fat:blood partition coefficients. To understand the determinants of the pharmacokinetics of D4, a multi-route physiologically based pharmacokinetic (PBPK) model has been developed to characterize the retention, distribution, and elimination of D4 following inhalation, dermal, oral and intravenous (IV) exposures in the rat. Concentrations of D4 in plasma, D4 exhalation rates, and elimination rates of D4 metabolites in urine, measured following inhalation, dermal, oral or IV dosing, form the basis for the model. In addition to liver, lung, blood, richly and poorly perfused tissues; the PBPK model contains deep-compartments for the liver and lung, a blood-lipoprotein pool, and multiple fat compartments. The PBPK model only provided good fits to pharmacokinetic data from the multiple routes of exposure when the dose routes were treated as if the D4 entered the circulation in different pools for the different routes. The disposition of D4 following both inhalation and dermal exposure were similar and appear to be governed by direct delivery of free molecular D4 into the blood. In contrast, disposition of D4 following oral exposure appears to be governed by uptake into the blood-lipoproteins and subsequent sequestration in various lipid pools. Similarly, pharmacokinetics following IV administration was also best described by a model structure with delivery of D4 directly into the deep blood compartment, indicating that the microemulsion form of D4 never becomes readily available as a free form of the compound. Unexpectedly the physical form of D4 delivered varied among dose routes. These dose route-route differences in pharmacokinetics need to be considered in human exposure assessments and in Dose-Response modeling. Supported in part by Silicones Environmental, Health and Safety Council of North America.

241 HUMAN INHALATION PHARMACOKINETICS OF OCTAMETHYLCYCLOTETRASILOXANE (D4): EVALUATION WITH A PHYSIOLOGICAL MODEL.

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D4, a lipophilic, volatile liquid, is used as a reaction intermediate and is found in industrial and consumer products. Workers and consumers may be exposed to D4 by inhalation or dermal routes. A human inhalation pharmacokinetic (PK) study (Toxicology Sciences, 44:206-13, 1998) and a physiologically based pharmacokinetic (PBPK) model for D4 inhalation in rats (Toxicology Sciences, 60:214-31, 2001) were recently published. The rat PBPK model showed that D4 is eliminated rapidly from the body after exposure due to its low blood:air partition coefficient, Pb (~ 1), and its relatively high metabolic clearance. Some D4 was present in blood in a form that was not readily exhaled, presumably bound to lipids. From the human PK study, data from 12 subjects exposed by inhalation to 10 ppm D4 for 1 hour with alternating periods of rest and exercise were analyzed with the PBPK model. The human data required a Pb of about 0.9. Human metabolism and fat partitioning constants remain to be determined more accurately (i.e., they could not be estimated from the data). When values for these parameters were based on those of the rat study, the model matched observations in the human PK study very well. The PBPK model results were also consistent with the decreased retention with increased alveolar ventilation seen in humans. While the human data set is less extensive than the rat data set, similar PK behaviors were noted including rapid clearance by exhalation and longer persistence in plasma than expected based on the Pb and the rate of metabolism. Many of the conclusions reached in PBPK modeling of D4 inhalation kinetics in rats appear valid for human kinetics of D4. Thus, a similar model structure, with a key feature of D4 sequestration in blood lipids, is required in both species. Supported in part by Silicones Environmental, Health and Safety Council of North America and by NIEHS T32 ES07321 for M. Reddy.

242 ASSESSING THE METABOLISM OF HEXAMETHYLDISILOXANE USING GAS UPTAKE METHODS AND PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING.

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Gas uptake methods have been successfully used to assess the metabolism of a wide variety of volatile organic chemicals. The technique works best with compounds of moderate blood:air and relatively low fat:blood partitioning, and little propensity to adsorb onto chamber surfaces. We extended these techniques to evaluate the *in vivo* metabolism of hexamethyldisiloxane (HMDS), a low molecular weight volatile siloxane. The studies utilized a static atmosphere closed chamber system designed by Filser and colleagues (Arch. Toxicol. 42: 123-136, 1979). Soda lime, used to prevent accumulation of exhaled CO_2 in the system, adsorbed a substantial part of HMDS thus limiting the interpretation of the kinetic data. Soda lime was replaced

by 3.5N NaOH providing acceptable background loss rates of less than 2% per hour. After resolving these technical issues, a series of gas uptake experiments were initiated to evaluate the metabolism of HMDS. In each experiment, two rats (Fischer 344, male, 8-9 weeks old) were exposed to initial concentrations of HMDS ranging from 500 to 5000 ppm and the chamber concentration was monitored over a period of 8 hr. Families of curves for rates of loss of HMDS from the chamber atmosphere were modeled with a four-tissue PBPK model. Excellent fits were obtained incorporating a single saturable metabolic pathway (Vmax=98 μM/hr; Km=86 μM) restricted to the liver. Blood:air and fat:blood partition coefficients were 1.5 and 230. The availability of a PBPK model for HMDS will provide the means to quantitatively characterize the retention, distribution, metabolism and elimination of this material, and support risk assessment extrapolations across dose, dose route, and species. Supported in part by Silicones Environmental, Health and Safety Council of North America.

243 PBPK MODELS OF KEPONE, MIREX, HEXAFLUOROACETONE AND ACETONE SUGGEST BINDING TO HEPATIC TISSUE.

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The structures of the pesticides Kepone® and mirex differ only by the presence of a ketone group in Kepone in place of two chlorines in mirex. Both compounds are extremely lipophilic, with log Kow values of 5 to 6. In spite of their physicochemical similarities, their distribution in the body differs markedly: Kepone concentration is highest in the liver; mirex concentration is highest in fat. We have noted a similarly anomalous distribution for hexafluoroacetone (HFA) and acetone: acetone distributes evenly throughout body tissues; HFA accumulates in liver. These two compounds are equally hydrophilic, having low Kow values of 0.6 (HFA) and -0.24 (acetone). Neither Kepone nor HFA is metabolized in rat. Physiologically based pharmacokinetic models have been designed to examine factors contributing to unusual distribution of Kepone and hexaflouroacetone compared to their respective counterparts. The Kepone/mirex model was constructed using blood, fat and liver concentrations of mirex in monkeys, determined after intravenous or oral administration of pesticide (Drug Metab. Dispos. 4:281, 1976). Blood:tissue distribution ratios calculated from the data determined distribution among tissues. Blood, fat and liver concentrations of Kepone in rats, determined after oral administration (Drug Metab. Dispos. 6:91, 1978) were effectively represented by this model only when liver binding parameters for maximal binding (Bmax) and binding affinity (Kd) were 5000 nmol/liver and 0.1 μM. Similarly, the HFA/acetone model was constructed using partition coefficients and metabolic enzyme constants appropriate for acetone (Occup. Environment Med.. 52:344, 1995). Blood and tissue concentrations of HFA in rats following subcutaneous dosing (Toxicol. Appl. Pharmacology 73:23, 1984) were adequately modeled with Bmax = 1200 nmol/liver and Kd = 18.1 μM. Liver sequestration may be a common characteristic for ketones with electron withdrawing substituents. (Supported by NIEHS grants ES05901 and P42 ES05949.)

244 DOSE DEPENDENT RATES OF MANGANESE (Mn) UPTAKE AND BILIARY ELIMINATION: COMPARTMENTAL ANALYSIS OF DIETARY AND GAVAGE DOSING STUDIES IN THE RAT.

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Tissue concentrations of the essential element Mn are regulated predominantly by control of gastrointestinal uptake and biliary elimination. Understanding of the comparative uptake, elimination and tissue distribution of Mn following oral or inhalation exposure to Mn is central to the development of quantitative tools for risk assessment of inhaled Mn. A three-compartment pharmacokinetic model consisting of the gut lumen, gut tissues and the liver was used to examine dose dependencies for absorption and elimination by simulating several dietary or gavage exposure studies in rats. Fitted rate constants provided excellent fits to true and apparent uptake of Mn following dietary exposure at concentrations of 1.5 μg/g and 100 μg/g. The first order rate constants for uptake decreased by a factor of 20 and the first order elimination rate increased approximately 100 fold across the dose range; the rate constant for elimination tended toward saturation at dietary levels greater than 35 μg/g Mn. The model was then compared with two additional dietary exposure studies, and provided good fits to reported bile elimination rates and liver concentrations. Combined dietary (45 μg/g) and oral gavage (0.2-10 mg Mn) appeared to exceed the capacity of uptake control, resulting in compensatory increases in elimination. These empirical representations of the important control processes for regulation of tissue Mn concentrations are necessary for incorporating dose-dependencies into physiologically based pharmacokinetic models describing tissue disposition following combined oral and inhalation exposure to Mn or Mn-containing byproducts of combustion. This work was supported by Ethyl Corporation.

245 SPECIES SPECIFICITY OF PLASMA PROTEIN BINDING OF TRICHLOROACETIC ACID AND ITS EFFECT ON TISSUE BIOAVAILABILITY USING PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING.

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Exposure to trichloroacetic acid (TCA) is widespread, either as a by-product of drinking water purification or as a metabolite of chlorinated solvents such as tri- and perchloroethylene. TCA has been shown to cause liver tumors in mice and is considered a probable human carcinogen. Physiologically-based pharmacokinetic (PBPK) modeling has important utility in examining species-related differences in chemical metabolism and distribution. A better understanding of the role of plasma protein binding for important metabolites like TCA and its influence on their bioavailability to liver and other tissues is needed for human risk assessment. However, there is a paucity of data. Mathematical expressions for plasma protein binding of TCA in several species were generated experimentally and included in PBPK models to predict species-specific differences in the bioavailability of TCA to the liver. Plasma protein binding of TCA in mouse, rat, and human plasma was determined *in vitro* using equilibrium dialysis. The resulting binding parameters and subsequent mathematical expressions of free and bound fractions of TCA were incorporated into PBPK models for each species. Information on the free fraction proved useful in making more accurate model predictions of liver bioavailability over a wide range of TCA plasma concentrations. Comparisons of species-specific differences in liver bioavailability as a function of TCA plasma concentration were thus demonstrated to be feasible, which can have significant utility in using PBPK modeling to assess human health risks. (This research was funded by Department of Energy Grant # DE FC02 98CH10902)

246 PHARMACOKINETIC MODELING OF THE PHYTOESTROGEN GENISTEIN IN RATS: SIMULATING THE LAG IN BILIARY EXCRETION.

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Phytoestrogens are endocrine-active compounds naturally occurring in some food crops, resulting in human dietary exposures. The phytoestrogen genistein, a significant component of this exposure, is mainly found in soy foods with high concentrations in tofu and soy infant formula. Genistein is believed to have a range of effects, from possible breast and prostate cancer prevention in humans to observed reductions in weight and ano-genital distances of rodents at birth. We seek to develop physiologically based pharmacokinetic (PBPK) models to estimate the internal dosimetry of genistein in rats and humans. Dosimetric data from the literature indicate that genistein undergoes enterohepatic circulation and that there is a genistein concentration-dependent lag in biliary excretion. The ordinary differential equations used in typical PBPK models are unable to capture the dynamics of this observed delay. In this research effort, we developed state-dependent delay differential equations (DDE) to model the lag in genistein biliary excretion. The values of the unknown kinetic parameters were obtained by fitting the model to the biliary excretion data. Model predictions of the data using the resulting optimized parameters are better than predictions without using the DDE. However, though the delay observed in the data is present in the DDE model simulations, the model does not adequately reproduce the true dynamics. Therefore, options for developing a more biologically motivated model to capture the delay dynamics more accurately will be explored. Such work may lead to insight into the underlying biological mechanism of the delay, which is currently unknown. These developments allow for quantification and extrapolation of any exposure-response relationships observed in experimental studies of genistein. Further, these models may be used as templates for modeling compounds with similar pharmacokinetics. This research was supported in part by the American Chemistry Council.

247 PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING OF DICHLOROACETIC ACID.

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Halogenated acetic acids (HAs) are by-products of drinking water disinfection. Dichloroacetic acid (DCA) is a commonly identified by-product that causes liver tumors in rodents. Because of the possible link to human cancer, EPA has set standards permitting a combined total of 60 mg/L of five common HAs in drinking

water with the eventual goal of eliminating DCA from drinking water under stage I regulations. Thus, understanding and predicting the pharmacokinetics of HAs associated with drinking water disinfection is critical to assessing associated risks to the public. Recently, we characterized the low dose pharmacokinetics and oral bioavailability of DCA in F344 rats. Important features of DCA kinetics are: the pronounced non-linearity in its elimination and the complex plasma-time profiles observed after oral dosing with secondary peaks appearing well after the initial absorption phase. The secondary peaks were not caused by enterohepatic recirculation (bile flow independent). The elimination of DCA is controlled primarily by liver metabolism, mediated through a novel class of GST isoenzyme, GST-zeta. An interesting feature of the GST-zeta pathway is the loss in enzyme activity and decreased metabolism that occurs following exposure to DCA. To aid in the risk assessment of HAs, we have been developing a physiologically based pharmacokinetic (PBPK) model for DCA that incorporates key aspects of DCA disposition identified from past experimental studies using both naïve rats and rats treated to deplete GST-zeta enzyme levels. The DCA model to be presented includes a multi-compartment description of the gastrointestinal system to account for the observed discontinuous absorption of DCA. The model also describes liver metabolism using the dispersion model that is better able to account for the saturable metabolism and resultant non-linearity in DCA elimination. Model predictions were validated against previously characterized plasma-time profiles for DCA and selected tissue concentration-time profiles obtained for this study. Supported by EPA STAR grant R825954.

248 DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR CHLOROBENZENE IN F344 RATS.

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Chlorobenzene is an industrial solvent used in the manufacture of pesticides, resins, inks, and paints. To better understand the dose-dependent and route-specific kinetics of chlorobenzene, this work focused on development and validation of a physiologically based pharmacokinetic (PBPK) model to describe the absorption, distribution, metabolism and elimination of chlorobenzene in rats. Partition coefficients were experimentally determined in rat tissues and blood samples using a vial equilibration technique. These solubility ratios were in agreement with previous reports. The *in vivo* metabolism of chlorobenzene was evaluated using groups of 3 F344 male rats exposed to 80-6750 ppm chlorobenzene in a closed, recirculating gas uptake system. Michaelis-Menten metabolic constants were optimized from the family of gas uptake curves using the PBPK model, and indicated a high affinity (Km) and high capacity (Vmax) for chlorobenzene metabolism. At the highest chamber concentration, the uptake curve could not be modeled without the addition of a first-order (Kfo) metabolic pathway. Pretreatment of animals with pyrazole, an inhibitor of oxidative microsomal metabolism, completely abolished uptake. The completed PBPK model was validated against real-time exhaled breath data collected from rats administered 400 mg/kg chlorobenzene in corn oil by oral gavage or intraperitoneal (IP) injection. Absorption rates were optimized from the exhaled breath profile from one animal per exposure route, and used to simulate the remaining data sets. Development of a comprehensive PBPK model for chlorobenzene in rats is the first step toward future extrapolations to apply to humans (Supported by the US Department of Energy under Contract DE-AC06-76RLO 1830).

249 DEVELOPMENT OF A PBPK MODEL FOR METHYL ETHYL KETONE IN F344 RATS.

K. D. Thrall, J. J. Soelberg, K. K. Weitz and A. D. Woodstock. *Molecular Biosciences, Battelle, Pacific Northwest Division, Richland, WA.*

Methyl ethyl ketone (MEK) is an industrial chemical commonly encountered in a wide variety of paint products. A PBPK model to describe the absorption, distribution, metabolism and elimination of methyl ethyl ketone (MEK) in rats was developed. Partition coefficients were experimentally determined in rat tissues and blood samples using a vial equilibration technique. These solubility ratios were in agreement with previous human-based estimates that MEK is uniformly soluble within all tissues. Both saturable (Km = 0.63 mg/L; Vmax = 5.44 mg/hr/kg body weight) and first-order (Kfo = 4.1 hr-1) metabolic rate constants for rats were derived from the optimization of a series of *in vivo* gas uptake curves conducted at various initial chamber concentrations. Pretreatment with pyrazole, an inhibitor of oxidative microsomal metabolism, decreased the slope of the uptake curve, but did not abolish metabolism. Optimal model fit to the gas uptake curve from pyrazole-pretreated animals was achieved with Km increased 50 times the value determined from naïve rats. The completed PBPK model was validated against real-time exhaled breath data collected from rats administered an intravenous (IV) injection of MEK. Model simulation of the IV-treated animals required alveolar ventilation to be reduced 30% in order to match the data. Exhaled breath profiles from animals treated with MEK by oral gavage or intraperitoneal (IP) injection exposures were evaluated, and absorption rates determined. Development of a comprehensive PBPK model for MEK in rats is the first step toward future extrapolations to apply to humans (Supported by NIOSH 1-RO1-OH03658-01A2).

250 PBPK MODEL FOR THE EFFECT OF GESTATIONAL EXPOSURE TO PERCHLORATE ON IODIDE KINETICS IN THE RAT AND EXTRAPOLATION TO HUMAN GESTATION.

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Perchlorate (ClO_4^-) disrupts endocrine homeostasis by competitively inhibiting the transport of iodide (I^-) into the thyroid. Therefore, the possibility of developmental effects from ClO_4^- induced iodide deficiency during gestation is cause for concern. Fetal development relies on maternally supplied I^- for hormone synthesis within the fetal thyroid. Though ClO_4^- passes through the placenta, the extent of inhibition in the fetus is unknown. A physiologically-based pharmacokinetic (PBPK) model was developed in the pregnant rat to predict ClO_4^- exposure and the resulting effect on iodide kinetics. Tissues with active uptake were described with Michealis-Menten (M-M) kinetics to simulate saturable transport. Physiological and kinetic parameters were obtained from literature and experiment. Systemic clearance, placental-fetal transport and M-M uptake parameters were obtained by fitting simulations to experimental data in the pregnant and fetal rat. The rat model is able to predict iodide kinetic data in the dam and fetus over 5 orders of magnitude (0.36 to 33, 000 ng/kg $^{131}I^-$), ClO_4^- distribution over 3 orders of magnitude (0.01 to 10 mg/kg-day ClO_4^-), and inhibition of maternal thyroid I^- uptake. The successful rat model served as the basis for a description of the corresponding human kinetics. Physiological parameters and species kinetic differences were obtained from literature and previously developed adult male rat and human ClO_4^- models. Kinetic parameters were scaled allometrically from the rat gestation model, except the parameters with significant species differences, which were obtained from the male human model and literature. The resulting model successfully describes maternal serum, urine, placenta, and thyroid, as well as fetal thyroid, kidney, stomach, serum and total fetal iodide in the human from several studies through gestation (weeks 6 to 40).

251 EVALUATION OF THE POTENTIAL IMPACT OF AGE- AND GENDER-SPECIFIC PHARMACOKINETIC DIFFERENCES ON TISSUE DOSIMETRY.

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Physiologically based pharmacokinetic (PBPK) modeling provides a means for quantitatively determining the impact of age- and gender-related changes in pharmacokinetics on chemical risk estimates. Specifically, these models provide a quantitative structure for estimating the effect of pharmacokinetic variability on the relationship between environmental exposures and biologically effective target tissue exposures. Three case studies were performed to support the development of a methodology that incorporates PBPK modeling to assess the likelihood that a chemical or class of chemicals may present an age- or gender-specific risk. The first case study focused on physiological and biochemical determinants of systemic toxicity, and tested the hypothesis that many of the pharmacokinetic differences observed across age and gender may be attributed to natural changes in physiological properties. The second case focused on the impact of differences in lung morphology and ventilation rate on both local and systemic toxicity as a function of the properties of the chemical. The third case study focused on the perinatal period, using PBPK models to evaluate *in utero* exposure *via* placental transfer *versus* exposure *via* lactational transfer to demonstrate critical periods of exposure from a pharmacokinetic perspective. These studies were exercised using parameters representative for multiple classes of chemicals to provide information on pharmacokinetic behaviors for each class of chemical. In general, variations in pharmacokinetic dose metrics for a chemical over a lifetime were within a factor of 2; however, in early childhood, exceptions were observed in which the dose metrics varied by approximately an order of magnitude. This work was supported by the Risk Assessment Methods Technical Implementation Panel of the American Chemistry Council.

252 ASSESSING DOSE- DEPENDENT GSH DEPLETION AND CHLOROTRIAZINE METABOLITE DISPOSITION AFTER ATRAZINE TREATMENT WITH A PHYSIOLOGICAL MODEL.

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Atrazine (ATRA), a water-soluble chlorotriazine herbicide, is used throughout the United States. High doses of ATRA impair the pre-ovulatory LH surge in female Sprague-Dawley rats, presumably due to actions in the hypothalamus. This response is related to ATRA and its three chlorinated, dealkylated CYP450 metabolites. Non-chlorinated triazines appear inactive. Both ATRA and the dealkylated metabolites undergo GSH conjugation with elimination of processed conjugates in feces and urine. We have modified a physiologically based pharmacokinetic (PBPK) model developed for radiolabeled disposition studies with atrazine (McMullin et al., The Toxicologist, 60, 150) to describe blood time-course of individual chlorinated triazines measured after derivatization (see Cranmer et al., this meeting) and to include the depletion of GSH during their metabolism. To evaluate GSH depletion, we used model predictions to design an experiment in which groups of rats received very high doses of these compounds, 800 mg ATRA/kg in 1% methylcellulose, or similar doses of the dealkylated metabolites or di-amino-chloro-triazine (DACT). Rats were sacrificed at 8 hours - the predicted time for maximal GSH depletion from the original model for radiolabeled ATRA. Liver, brain and various other tissues were analyzed for GSH concentrations and blood for individual chlorotriazines. Unexpectedly, DACT caused significant GSH depletion in liver (3.8 ± 0.2 mM), compared to control rats (5.2 ± 0.3 mM), but no depletion was observed at this time with ATRA (5.2 ± 0.4 mM). These apparent differences among these chlorotriazines in GSH reactivity, now incorporated into the PBPK model, emphasize the value in refining the original radiolabeled ATRA PBPK model to accurately account for the formation and especially for the GSH-related clearance of all four chlorotriazines. This research was funded by EPA STAR grant (R-828610-01-0).

253 THE EFFECTS OF SMOKING ON HUMAN HEALTH.

K. Rodgers and B. Freed. *Ob/Gyn, USC, Los Angeles, CA.*

Recent court settlements between the cigarette industry and state health agencies has renewed interest in the effects of smoking on human health. While the association between smoking and lung cancer has been known since the 1930s, only recently have the immunosuppressive effects of smoking been appreciated. Smoking represents the most potent and widespread immunosuppressive agent with human exposure. Both marijuana and tobacco smoking render patients more susceptible to respiratory tract infections. Certain inflammatory lung diseases, such as hypersensitivity pneumonitis and sarcoidosis, are actually attenuated by the immunosuppressive effects of smoking. Recent studies have clearly identified several immunosuppressive compounds in smoke that act locally (hydroquinone, catechol, acrolein and crotonaldehyde) or systemically (cannabinoids and nicotine). This symposium will explore the molecular mechanisms by which these components of smoke affect signaling pathways, gene expression and cell cycle progression in T lymphocytes and neutrophils.

254 INHIBITION OF G_1 AND S PHASE T CELL CYCLE EVENTS BY CIGARETTE SMOKE.

B. M. Freed. *Allergy and Clinical Immunology, Univ Colorado Health Sciences Center, Denver, CO.*

Cigarette smoke is a potent immunosuppressive agent that completely blocks T and B lymphocyte responses in the lungs. We have identified several compounds in both the tar and gas phases that inhibit distinct events associated with T cell activation and proliferation. Hydroquinone (HQ), catechol and phenol represent the three major phenolic components in cigarette tar. They are present in cigarette smoke at concentrations that are directly proportional to tar content. Hydroquinone and catechol inhibit lymphocyte proliferation by interfering with two different stages of the cell cycle. When added to resting T cells prior to mitogenic activation, they permit many early activation events, such as IL-2 production, expression of CD25 and CD69. However, they cause an aborted blastogenesis by blocking progression from G_1 to S phase of the cell cycle. This phenomenon is associated with inhibition of Rb and transferrin receptor expression. When added to proliferating lymphocytes, HQ and catechol cause an immediate cessation in DNA synthesis by quenching the tyrosyl radical in ribonucleotide reductase. Phenol has no effect on any of these events. Acrolein and crotonaldehyde are thiol-reactive α, β-unsaturated aldehydes present in the gas phase of cigarette smoke. Unlike HQ and catechol, they are present even in ultra low-tar cigarettes. At doses found in a single cigarette, they inhibit the production of several proinflammatory cytokines, including IL-1β, IL-2, TNF-α and IFN-γ. The immunosuppressive effect can be completely abrogated by N-acetylcysteine, suggesting that they function by reacting with thiols. Acrolein and crotonaldehyde inhibit cytokine production by blocking NF-κB and AP-1 DNA binding activity, thereby preventing expression of genes that are regulated by these transcription factors.

255 EVIDENCE FOR CB1/CB2-DEPENDENT AND -INDEPENDENT MECHANISMS OF CANNABINOID-MEDIATED MODULATION OF LEUKOCYTE FUNCTION.

N. E. Kaminski, B. F. Kaplan, T. R. Jan and G. K. Rao. *Michigan State University, East Lansing, MI.*

Over three decades of studies have unequivocally demonstrated that the immune system is sensitive to modulation by cannabinoids, a family of structurally-related congeners derived from the marihuana plant. In fact, marihuana smoke contains over 60 different cannabinoids, the majority of which have not been investigated for biological activity. For those cannabinoids that have been investigated, the mechanism responsible for their biological activity remains poorly understood. Their effects on the two primary target organs, the central nervous system and the immune system appear to be mediated, at least in part, *via* G-protein coupled cannabinoid receptors, CB1 and CB2. A major focus of this laboratory has been the elucidation of the molecular mechanism by which cannabinoids modulate the T cell-derived cytokines, interleukin (IL)-2 and IL-4. One major signal transduction pathway we have recently identified that is disrupted by cannabinoids and which is critical for T cell function is the calcium-mediated activation of the nuclear factor of activated T cells (NF-AT). NF-AT DNA binding, which is essential for the transcriptional regulation of IL-2 and a number of other T cell-derived cytokines, is disrupted by immunosuppressive cannabinoids. Cannabinoid treatment was also found to induce a rapid and sustained rise in $[Ca^{+2}]_i$ which was concentration responsive and of a magnitude similar to that produced with 500 nM ionomycin. The rise in $[Ca^{+2}]_i$ by cannabinol and Δ^9-tetrahydrocannabinol was attenuated by pretreatment of splenocytes with the CB1 (SR141716A) and CB2 (SR144528) antagonists, in combination, and in thymocytes with SR144528 alone. Interestingly, neither SR141716A nor SR144528 alone or in combination attenuated cannabinoid-mediated inhibition of IL-2 gene expression. Collectively, these studies suggest that cannabinoid-mediated modulation of IL-2 is mediated in part *via* a disruption of $[Ca^{+2}]_i$ through a cannabinoid receptor-dependent mechanism as well as through cannabinoid-receptor-independent pathways.(Supported by NIH Grant DA07908).

256 NEUROIMMUNE COMMUNICATION IN NICOTINE-INDUCED IMMUNOSUPPRESSION.

M. L. Sopori. *Respiratory Immunology, Lovelace Respiratory Research Institute, Albuquerque, NM.*

Chronic exposure of rats and mice to nicotine (NT) decreases the antibody-forming cell response and Con A-induced proliferation of T cells. This immunosuppression is accompanied by impaired antigen receptor-mediated signaling in T cells, including constitutive activation of Fyn and other protein tyrosine kinases. Similar changes in the immune response were also observed after intracerebroventricular administration of NT, which were reversed by simultaneous administration of the nicotinic acetylcholine receptor (nAChR) antagonist, mecamylamine, indicating that NT affected the immune system *via* the nAChRs in the central nervous system. The immunosuppressive effects of NT were also seen in adrenalectomized rats, but not in animals pretreated with ganglionic blockers. Thus, the effects of chronic NT exposure on the immune system are independent of the hypothalamus-pituitary-adrenal axis but dependent on the autonomic nervous system. Surgical sympathetic denervation of the spleen or treatment of animals with the muscarinic antagonist, atropine, did not reverse the effects of NT on the immune system. Therefore, it is likely that the parasympathetic but not the sympathetic pathway transmits signals from the brain to nAChRs on T cells. In this regard, our recent data suggest the presence of α7-containing nAChRs on T cells. Interestingly, following chronic NT treatment, animals exhibited enhanced expression of IL-1βmRNA in the brain that was kinetically correlated to the induction of immunosuppression. Moreover, unlike the control wild-type mice, IL-1β receptor KO mice did not exhibit immunosuppression in response to NT exposure. These results suggest that the neuroimmune effects of NT are mediated through the parasympathetic pathway and that IL-1β may play an important role in the neuroimmune modulation by chronic NT treatment. Supported in part by grants from the NIH (DA04208, DA04208-13S1, HD38222).

257 MODULATION OF NEUTROPHIL REACTIVE OXYGEN SPECIES PRODUCTION BY CIGARETTE SMOKE EXTRACT.

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The production of reactive oxygen species (ROS) by neutrophils appears to play a central role in the pathogenesis of cardiovascular and respiratory diseases induced by cigarette smoke (CS). In particular, neutrophils initiate and maintain the inflammatory process, primarily *via* their ability to generate ROS. However, a number of studies have produced conflicting results in regard to the effect that CS has on neutrophil ROS production. Furthermore, there are data that suggest that CS may prime neutrophils resulting in enhanced ROS production following exposure to an inflammatory mediator such as tumor necrosis factor-α (TNF-α). We have evaluated the effect of varying concentrations of CS extract on neutrophil ROS production and priming followed by exposure to inflammatory mediators (e.g., interleukin-1) known to be elevated in cigarette smokers. Data from our work will be presented.

258 NEURORECEPTORS AS THE BASIS FOR INSECTICIDE RESISTANCE AND SELECTIVE TOXICITY.

T. Narahashi. *Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL.*

The resistance of insects to insecticides and the selective toxicity in mammals and insects are among the most striking and important features of insecticides. Insecticide resistance is one of the most serious problems in using and developing insecticides, and their selective toxicity is one of their most important characteristics. Whereas diverse mechanisms underlie these two different features of insecticides, many, if not all, such cases have been shown to be ascribed to factors at the target site, specifically the sensitivity of target neuroreceptors to insecticides. There have been profound developments in the cellular and molecular mechanisms that underlie these phenomena during the past several years owning to the development of advanced eletrophysiological, molecular biological and genetic techniques. For example, insect resistance to dieldrin and the pyrethroids is due in many cases to the markedly reduced sensitivity of GABA receptors and sodium channels, respectively, which are in turn ascribed to mutations of single amino acids in the target receptor/channel. Similarly, the selective toxicity between mammals and insects is also due to differential target site sensitivities as has been shown for the pyrethroids.

259 POINT MUTATIONS IN THE INSECT PARA SODIUM CHANNEL ALTER THE CHANNEL SENSITIVITY TO PYRETHROIDS.

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Pyrethroid insecticides are used worldwide to control insect pests of agricultural and medical importance. Pyrethroids exert toxic effects on insects by binding to a unique, but undefined site on the voltage-gated sodium channel and thereby altering sodium channel gating properties. Multiple sodium channel point mutations have been found in several pyrethroid-resistant insect species. The mutations, located in IS6, IIS6, the linker connecting IIS4-S5, and the linker connecting the domains I and II, have been shown to reduce the sodium channel sensitivity to pyrethroids. In this talk, I will review the effects of these sodium channel mutations on the gating and pyrethroid-sensitivity of the German cockroach Para sodium channel. In addition, we recently identified two Para alternative splice variants, which differ greatly in their sensitivities to pyrethroids. Further analysis of these two splice variants will reveal additional amino acid residues involved in sodium channel interaction with pyrethroids.

260 INSECTICIDE RESISTANCE: OLD PROBLEMS OR NEW SOLUTIONS?

R. H. ffrench-Constant. *Biology and Biochemistry, University of Bath, Bath, United Kingdom.* Sponsor: T. Narahashi.

Overuse of insecticides has often led to resistance in insect populations. Our laboratory has focused on how an understanding of the molecular basis of resistance can a) help us understand how resistance has evolved, b) help us identify the receptors/enzymes involved and c) help us understand if resistance can be avoided, or at least delayed, in the future. We will discuss two specific examples, resistance to cyclodienes and fipronil mediated *via* an altered GABA receptor gene (Resistance to dieldrin or Rdl) and P450 mediated resistance to DDT and the neonicotinoids. In each case we will examine central questions. 1) How many genes are involved? 2) What mutations are involved? 3) How have these mutations spread in natural populations? 4) What levels of cross-resistance are conferred by these genes and to what compounds? 5) Will resistance go away if we stop spraying? 6) Can we predict resistance to novel compounds that are yet to come to market? These questions will be examined both in an academic (receptor biology and resistance evolution) and industrial (implications for the design and introduction of new compounds) context.

261 NEONICOTINOID INSECTICIDES: MECHANISM OF SELECTIVITY FOR INSECT *VERSUS* MAMMALIAN NICOTINIC RECEPTORS.

M. Tomizawa and J. E. Casida. *Department of Environmental Science, Policy and Management, Environmental Chemistry and Toxicology Laboratory, University of California, Berkeley, Berkeley, CA.* Sponsor: T. Narahashi.

The last decade opened the era of neonicotinoids (represented by imidacloprid) as a major new class of highly effective and widely used insecticides for crop protection and veterinary pest control. The favorable selective toxicity of neonicotinoid insecticides for insects *versus* mammals is not shared by their *N*-unsubstituted imine metabolites and analogs or by nicotine or epibatidine. The same selectivity profile is evident at the target site level, i.e., the insect nicotinic acetylcholine receptor (nAChR) *versus* mammalian nAChR subtypes (α1, α3, α4 and α7). The insect-selective compounds are not protonated with a nitroimine, cyanoimine or nitromethylene substituent and the mammalian-selective compounds are ionized at physiological pH. We propose that the negatively-charged tip of the nitro or cyano group interacts with a putative cationic subsite of the insect nAChR. This contrasts with the mammalian nAChR subtypes where the iminium cation of *N*-unsubstituted imine metabolites or analogs of neonicotinoids or ammonium nitrogen of nicotine and epibatidine interacts with the anionic subsite. Further, only minor structural changes in neonicotinoids confer differential subtype selectivity for mammalian nAChRs. Chronic exposure to neonicotinoids, metabolites and analogs up-regulates the α4β2 nAChRs in M10 cells as initiated by receptor-compound interaction and followed by intracellular events.

262 TARGET SITE SELECTIVE TOXICITY TO INSECTICIDES IN MAMMALS AND INSECTS.

T. Narahashi. *Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL.*

Most insecticides are more toxic to insects than to mammals, and the selective toxicity of certain insecticides has been shown to be ascribed to the higher target site insecticide sensitivity in insects than in mammals. The major primary target of pyrethroids is the sodium channel, and the neuronal sodium channel of cockroaches were much more sensitive than that of rats. For example, allethrin was approximately 1000-fold more potent on the cockroach sodium channel than on the rat dorsal root ganglion (DRG) tetrodotoxin (TTX)-sensitive sodium channel based on the measurement of the percentages of sodium channels modified by the pyrethroid. Fipronil blocks the GABA receptor, and was more potent on the cockroach GABA receptor than on the rat DRG $GABA_A$ receptor. Indoxacarb and its metabolite DCJW have been shown to act on multiple target sites. Both blocked the sodium channels with a higher potency in cockroaches than in rats. They acted on the α7-type and α 4 β2-type nicotinic acetylcholine (nACh) receptors of rats in a complex manner: indoxacarb stimulated the α 4 β2 receptor but inhibited the α 7 receptor, while DCJW had no effect on the α4β 2 receptor with slight inhibition of the α7 receptor. The cockroach nACh receptor, which had characteristics different from either the rat α 7 or α4 β2 receptor, was inhibited by indoxacarb. A marked difference was found in the response of GABA receptors: the rat $GABA_A$ receptor was totally insensitive to indoxacarb or DCJW, whereas the cockroach GABA receptor was strongly inhibited by indoxacarb. In summary, the mechanisms of selective toxicity to pyrethroids, fipronil and indoxacarb reside in the differential sensitivity of target receptors between mammals and insects. Supported by NIH grant NS14143.

263 SELECTIVE ACTIONS OF INSECTICIDES ON LIGAND-GATED ION CHANNEL SUBTYPES.

V. L. Salgado. *Insecticide Biochemistry, Aventis CropScience, Frankfurt am Main, Germany.* Sponsor: T. Narahashi.

Nicotinic receptors and chloride-channel-gating GABA and glutamate receptors, all members of the cys-loop superfamily of ligand-gated ion channels, are important insecticide target sites. Recent electrophysiological studies on these receptors in insect neurons show the presence of subtypes of each of these receptor types, with large differences in sensitivity to insecticides and other drugs. Additionally, compounds that act allosterically on these receptors, as modulators or channel blockers, can act on more than one type of receptor in this superfamily. For example, the effects of avermectins on GABA receptors were studied long before the inhibitory glu-

tamate receptor were recognized as target sites, and more recently, fipronil, which has long been known to block GABA-gated chloride channels, has been found to also block glutamate-gated chloride channels. Similarly, spinosad, whose primary mode of action is modulation of nicotinic receptors, has also shown effects on GABA receptors. Thus, a complete understanding of the insect toxicology of these compounds requires an understanding of their interactions with a multiplicity of receptors. New comparative studies of the actions of neonicotinoids, spinosyns and fiproles on subtypes of nicotinic, GABA and Glutamate receptors help to clarify our understanding of the action of insecticides on these important target sites, and provide important new insights into resistance mechanisms and insect selectivity.

264 THE POTENTIAL FOR BIOLOGICAL MODELING TO IMPROVE CHILDREN'S RISK ASSESSMENT.

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Fundamental differences between animal and human development create unique challenges for assessing the risk of pharmaceutical or chemical exposures to children's health. This challenge can be met by integrating the basic biology of animal and human development with chemical-specific mechanisms or modes of action through the development of quantitative, biological models of target tissue dose and/or response. Starting from a single cell and growing over a billion-fold by birth, developing embryos can become targets for toxicity when the mother is exposed during specific windows of susceptibility. Neonatal exposures to certain chemicals continue to be mediated by the mother *via* lactation. Similar to the progress made in cancer risk assessments, biological models (physiologically based pharmacokinetic [PBPK] and biologically based dose-response [BBDR]) have increasingly been used to describe target tissue dose and response in animals during pregnancy and lactation. However, significant issues remain in the ability to extrapolate from animals to humans given the unique changes that occur in maternal, embryo, fetal and neonatal biology. Unlike most PBPK or BBDR models, models of pre- and postnatal development must deal with the dynamics of rapid growth (maternal and embryonic), changes in the state of differentiation of developing structures, uniquely expressed or uniquely functioning signal transduction or enzymatic pathways, and unusual routes of exposure (e.g., lactation). Our understanding of the dynamics of normal development at a molecular level, and of mechanisms of developmental toxicity, are increasing at a rapid pace, making BBDR models of developmental toxicity more feasible, although there are still important gaps in our knowledge. The symposium will lay out basic issues in, and examples of, PBPK modeling in the pregnant mother and fetus, and early childhood, along with progress and challenges in BBDR modeling of adverse events in structural and functional development.

265 PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING OF PRENATAL GROWTH AND EXPOSURES.

R. H. Luecke. *Chemical Engineering, Univ. of Missouri, Columbia, MO.* Sponsor: G. Daston.

Physiologically based pharmacokinetic (PBPK) models have recently been developed to include description of organ growth and physiological changes in the maternal and embryo/fetal environments. These pregnancy models include physiological data and growth equations for humans, monkeys, rats and mice and may be extrapolated to other species. Drug or chemical concentrations are calculated in both maternal and fetal organs. The models have also been partially extended with growth equations for neonates. Since accidental human exposure may involve more than one incident and more than one route, the model accommodates simultaneous and/or sequential exposures involving multiple routes. Following exposure to a single chemical, a second compound can be simultaneously modeled as a metabolite. Comparative simulations using pharmacokinetic data for methyl mercury as reported in the literature for 12 mammalian species were used to elucidate the impact of species-specific response differences. A mathematical approach is also outlined for use in conjunction with PBPK models to simulate a window of susceptibility for teratogenic sensitivity during pregnancy. The procedure is illustrated with an example based on literature data.

266 DEVELOPMENT OF BIOLOGICALLY BASED DOSE-RESPONSE (BBDR) MODELS FOR DEVELOPMENTAL TOXICITY.

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As a prototype BBDR model, we have developed a mechanistically-based model for the well-studied teratogen 5-FU, which is known to interfere with DNA synthesis *via* inhibition of thymidylate synthetase (TS) and consequently impede DNA synthesis and cell replication. Our model is based on the sequence: target (embryo) dose → TS inhibition → altered nucleotide (dNTP) pools → reduced DNA synthesis → reduced tissue growth → dysmorphogenesis. These events have been quantified experimentally in rat litters after maternal s.c. dosing with 0-40 mg/kg 5-FU on day 14 of gestation, which produces developmental toxicity including reduced fetal weight and limb defects. Measurements of dose-response for mechanistic endpoints include TS enzyme activity, embryonal nucleotide pool sizes, cell cycle distribution, DNA content, cell death, limb bud development and limb dysgenesis have been carried out, and quantitative linking of these endpoints indicates that the model based on TS inhibition can predict the Dose-Response for limb defects and fetal weight. A mathematical model linking TS inhibition to dNTP pool perturbations has been developed. Similar dosing with another TS inhibitor, the folate analog tomudex, produces most, but not all, of the effects of 5-FU, supporting the TS-based model. The severity of tomudex toxicity *in vitro* and *in vivo* are inversely correlated with embryonal folate supply.

267 PHARMACOKINETIC CONSIDERATIONS IN CHILDREN'S RISK ASSESSMENT.

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Pharmacokinetic processing of xenobiotics can differ widely between children and adults due to physiological differences and immaturity of enzyme systems and clearance mechanisms. This makes extrapolation of adult dosimetry estimates to children uncertain, especially at early ages. While there is very little PK data for environmental toxicants in children, there is a wealth of such data for therapeutic drugs. Using published literature, a Children's PK Database has been compiled comparing child and adult PK parameters for 44 drugs. This has enabled comparison of child and adult PK function across a number of cyp pathways and certain Phase II conjugation reactions and renal elimination. The across age group differences seen *in vivo* with drugs are consistent with *in vitro* liver bank studies and present a PK developmental profile relevant to environmental toxicants. Variability analysis points out key periods of rapid development where children's responses appear particularly heterogeneous. The manner in which this PK information can be applied to the risk assessment of children includes qualitative approaches (e.g., adjustment of uncertainty factors) and quantitative approaches (PBPK modeling). PBPK parameter values need to be specific to physical characteristics (body mass; compartment size and blood flow; water and lipid content; cardiac output and ventilation rate; partition coefficients) as well as to metabolic and renal elimination rate constants that pertain to children. Development of rate constants is aided by the Children's PK Database in that it provides case studies against which models can be calibrated and validated with respect to specific CYPs or other metabolism/ elimination pathways. Such validated children's models could then be used to adapt adult PBPK models for environmental toxicants to children and thus bring PBPK modeling into the risk assessment process for children. The views expressed are those of the author(s) and do not necessarily reflect the views or policies of the State of CT or USEPA.

268 MODELING ENDOCRINE ACTIVE COMPOUNDS (EACS) ACROSS LIFE STAGES.

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Questions about the potential for endocrine disruption have focused upon differential sensitivities across life stages and the potential that dose-response behaviors would vary across these periods. Some hormone-mediated changes in gene expression are "organizational" during development (e.g. establishing system set points) *versus* "activational" changes in adults (e.g. altering the current level of expression). Biological modeling of the impact of exogenous compounds on hormonally mediated events should ultimately address both pharmacokinetic and pharmacodynamic differences. Physiologically-based models of the pharmacokinetics of EACs during pregnancy, lactation, through puberty and into adulthood have accounted for factors such as changes in compartment size, blood flows, and serum binding proteins. Use of such models to estimate tissue dosimetry is dependent upon information about the mode of action including the window of vulnerability, the active form of the compound, and the biology of the endogenous signaling system. Models have been constructed for adult hormone-mediated processes, notably central axis function in rats and humans. Models of receptor-mediated processes have described *in vitro* and *in vivo* data, e.g. uterine receptor occupancy and positive feedback loops in regulatory phenomenon. While such models may help to understand the pharmacodynamics of EACs in adults, understanding their effects during *in utero* development may require describing morphogen-like gradients of endogenous hormone

as well as receptor-mediated responses to circulating hormones. These efforts at pharmacokinetic and pharmacodynamic modeling for EACs at different life stages are designed to assist in evaluating questions of dose-response behavior and cross-species extrapolation. (This abstract has been subjected to Agency review and approved for publication. LKP was funded by the EPA/UNC Toxicology Research Program, Training Agreement CT827206.)

269 PROGRESS IN DEVELOPMENT OF EMBRYOLOGICALLY BASED DOSE-RESPONSE MODELS.

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The goal of researchers working in the area of developmental toxicology is to prevent adverse reproductive outcomes (early pregnancy loss, birth defects, reduced birth weight, and altered functional development) in humans due to exposures to environmental contaminants, therapeutic drugs, and other factors. To best achieve that goal, it is important that relevant information be gathered and assimilated in the risk assessment process. One of the major challenges of improved risk assessment is to better use all pertinent biological and mechanistic information. This may be done qualitatively (e.g., demonstrating that the experimental model is not appropriate for extrapolation purposes); semi-quantitatively (using information to reduce the degree of uncertainty present under default extrapolation procedures), or quantitatively (formally describing the relationships between exposure and adverse outcome in mathematical forms, including components that directly reflect individual steps in the overall progression of toxicity). With particular reference to developmental toxicity, the quantitative models almost invariably encounter a "black box" in translating a particular biochemical perturbation into a morphological alteration. This presentation will review recent progress in generating dynamic models of tissue and organ development (e.g., the formation of somites which ultimately differentiate into the axial skeleton) that can one day be coupled with pharmacokinetic and biochemical models to fully understand the pathogenesis of adverse pregnancy outcomes.

270 INTRODUCTION TO THIOREDOXINS AND THIOREDOXIN REDUCTASES: CENTRAL ROLES IN TOXICITY AND CANCER.

L. P. Kehrer. *Pharmacology and Toxicology, The University of Texas at Austin, Austin, TX.*

Thioredoxins (Trx) and thioredoxin reductases (TrxR) are components of widely distributed and highly conserved thiol redox systems with diverse functions in redox signaling, detoxification, repair and proliferation. Both Trx and TrxR contain dithiol motifs, that undergo cyclical oxidation/reduction during catalytic turnover. These thiols are reactive with alkylating agents, making the proteins susceptible to inactivation by reactive electrophiles and oxidative stress. Trx expression is increased in response to oxidative stress and its distribution between cytoplasm and nucleus is altered in response to DNA damage. Mitochondria contain distinct forms of both Trx and TrxR. This initial overview will provide a brief review of the structure and enzymology of the system and make some comparisons to the glutathione system. As a whole, this symposium will provide a perspective on how Trx and TrxR are important to consider in terms of cellular responses to toxicologic insults.

271 STRUCTURE AND FUNCTION OF THE THIOREDOXIN-THIOREDOXIN REDUCTASE SYSTEMS.

A. Holmgren. *Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden.* Sponsor: J. Kehrer.

Thioredoxins are present in all cells and are the main disulfide reduction enzymes with a large number of functions. Reduction of the active site disulfide in thioredoxin (Trx) by NADPH is catalyzed by thioredoxin reductases (TrxR). Mammalian TrxR are very different from the small enzymes present in bacteria and have a broad substrate specificity including lipid hydroperoxides and selenite. The rat enzyme contains a glutathione reductase scaffold elongated by 16 residues and a C-terminal conserved sequence: Gly-Cys-Sec-Gly, where Sec is selenocysteine. We have expressed the rat enzyme in E.coli by changing the TGA codon for Sec498 to Cys or Ser, or a stop codon. The mutant enzymes were purified to homogeniety in high yield and contained one mol of FAD per subunit. Only the Sec to Cys mutant enzyme was active in reduction of thioredoxin with a 100-fold decrease in Kcat compared to the wild type Se-containing enzyme. The pH-optimum for the Cys mutant enzyme was 9 as opposed to 7 for the Sec-containing wild type enzyme, consistent with Se involvement in catalysis and explaining why this trace element is required for cell growth. The oxidized enzyme contained a unique selenenylsulfide bridge which is reduced to a selenolthiol in the reduced enzyme by electrons from the dithiol in the second subunit. The Cys mutant enzyme has been crystallized and the three-dimensional structure and the mechanism of the enzyme based on a 3 Å resolution map will be presented.

272 THIOREDOXIN IN CANCER DEVELOPMENT AND TREATMENT.

G. Powis. *Arizona Cancer Center, University of Arizona, Tucson, AZ.* Sponsor: L. Kehrer.

Mammalian thioredoxins (Trxs) are small redox proteins that undergo reversible oxidation and reduction by NADPH dependent selenocysteine containing flavoprotein thioredoxin reductases. Trx-1 is found in the cytoplasm, nucleus and is secreted by cells. Trx-1 reduces Cys residues on proteins including the transcription factors NF-kB and HIF-1alpha to increase their DNA binding and transactivating activity. Trx-1 also binds directly to proteins to regulate their activity including apoptosis signal regulating kinase-1 (ASK1) and the tumor suppressor PTEN. Nuclear targeted Trx-1 transforms cells. Trx-1 is also a potent cell survival factor and inhibitor of apoptosis. The stable transfection of mouse WEHI7.2 lymphoma cells with human Trx-1 inhibits apoptosis induced by a variety of agents. When inoculated into scid mice the Trx-1 transfected WEHI7.2 cells form tumors that grow more rapidly and show less spontaneous apoptosis than vector-alone transfected cells, and that are resistant to growth inhibition by dexamethasone. A catalytic site redox inactive mutant Trx-1 acts as a dominant negative to promote apoptosis of WEHI7.2 cells in culture and to inhibit MCF-7 human breast cancer tumor growth in mice. Trx-1 expression is significantly increased in a number of human primary cancers including lung, colon, gastric, cervix, liver, pancreatic and squamous cell cancer. Human primary colorectal cancer increased Trx-1 is associated with decreased patient survival. Trx-1 is a target for cancer drug development and PX-12, an inhibitor, of Trx-1, is soon to enter Phase I clinical trial.

273 REDOX OF THIOREDOXIN IN OXIDANT-INDUCED APOPTOSIS.

D. P. Jones and W. H. Watson. *Department of Biochemistry, Emory University, Atlanta, GA.*

Two major thiol-containing systems exist that modulate cellular redox balance as well as protect against oxidative stress; one utilizes the tripeptide glutathione (GSH) and the other uses the small protein thioredoxin (Trx). These systems have similar NADPH-dependent reductases and have overlapping functions and activities. Our recent research shows that the redox state of the GSH pool is maintained over a narrow range in cells and varies in association with cell proliferation, differentiation, redox signaling of gene expression and apoptosis. To determine whether the redox state of Trx similarly varies in association with changes in cell function, we have developed a Western blot technique that involves separation of oxidized and reduced forms of Trx based upon the carboxymethylation of thiols. Disulfides do not undergo carboxymethylation and consequently oxidized forms of Trx can be separated from reduced forms by PAGE under native conditions. Each form is detected by Western blotting using an antibody which detects both oxidized and carboxymethylated forms. Elution of the corresponding bands from the gels and analysis by MALDI-mass spectrometry has been used to confirm the identity of the reduced and oxidized Trx species. The results show that Trx is oxidized during oxidative stress and that redox varies independently of the GSH pool under different growth and toxicologic conditions. Application of the method to nuclear and cytosolic fractions further shows that redox in the nuclear compartment is distinct from that in the cytoplasm. (Supported by NIH grant ES09047).

274 REGULATION OF THIOREDOXIN-DEPENDENT PEROXIDASE PEROXIREDOXIN.

S. Rhee. *Laboratory of Cell signaling, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD.* Sponsor: D. Jones.

Recent evidence suggests that H2O2, produced in response to stimulation of a variety of receptors, serves as an intracellular messenger. Timely elimination of second messengers after completion of their mission is critical for receptor signaling.This would seem especially true for H2O2, which is readily converted to deleterious hydroxyl radicals. Peroxiredoxin (Prx)is a family of peroxidases that exist in multiple isoforms in a variety of cells. The four mammalian members, Prx I, II, III, IV, contain two conserved cysteines that correspond to Cys52 and Cys 173 of human Prx I. They exist as a head-to-tail dimmer. The oxidized intermediates of Prx I-IV pro-

duced in the presence of H2O2 are dimmers containing intermolecular disulfide between the two conserved cysteines and the disulfides are reduced by thioredoxin but not by glutaredoxin or glutathione. Cytosolic Prx enzymes (Prx I, II, and IV) participate in receptor signaling by eliminating H2O2. Prx enzymes may participate passively by removing H2O2 molecules that diffuse away from the sites of production or participate actively by being subjected to spatiotemporal regulation. Our recent studies suggest that the latter is likely the case. Prx I and II are mainly cytosolic but are also found in nuclear fractions. The amount of Prx I and II in the nuclear fraction increases in response to serum stimulation and decreases in response to treatment with H2O2 or UV. PrxI and PrxII, which contain a consensus site [TP(K/R)K] for phosphorylation by cyclin dependent kinase (CDK), are phosphorylated by several CDKs in cells. Phosphorylation by CDK abolished peroxidase activity. When experiments were carried out with HeLa and NIH3T3 cells synchronized at various cell cycles, the highest level of Prx I phosphorylation was detected in G2/M phase arrested with nocodazole. When the cells were released from nocodazole block, the levels of Prx I phosphorylation gradually decreased as the number of mitotic cells diminished. These results suggest that Prx I and II play important roles in regulating H2O2 concentration in nucleus.

275 THE FIELD OF TOXICOLOGY-NEW CENTURY, NEW CHALLENGES.

B. A. Schwetz. *Office of the Commissioner, FDA, Rockville, MD.*

The field of toxicology is driven by issues that relate to safety of consumer products, occupational exposures, human exposure from substances in the environment, as well as the effect of chemicals on environmental species. In the coming years, toxicology will face new types of issues, ones that will require new types of expertise to deal with them effectively. Although toxicology will continue to be central as the basis for making good decisions, we must plan ahead now so that we have the right types of expertise to make future decisions. How will new technology be different from the recent past? While large molecular weight substances are still composed of chemical units, there will be less emphasis in the future on traditional chemicals, particularly those of low molecular weight. We will be faced with high throughput, large volume data systems. This capability will present challenges to the field of toxicology that we have not experienced before. As we move away from traditional chemical-driven questions to issues related to products of new technology, some parts of our previous experience will be less useful than they have been in the past. As a consequence, our experience in traditional risk assessment will also be less valuable than in the past, partly because there will be no historical experience for many of the types of molecules that we will be dealing with in the future. With the anticipated change in toxicological issues, there are important questions about the future of toxicology. Will we as a field be prepared to handle these issues? Are we training scientists in areas to be responsive to these issues? How do we avoid shortages of scientists in certain critical areas and, perhaps, more important, how do we avoid creating an excess of scientists in less critical areas? Who will train people in these required areas of expertise? Most importantly, how will we attract these people to help us with questions in the field of toxicology? We need to begin to evaluate our strategy so that newly-trained toxicologists will be prepared to meet the future demands of our field.

276 IMMUNOLOGY/MICROBIOLOGY-BRIDGING A LINK TO TOXICOLOGY.

K. L. White. *Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA.*

One of the most challenging activities facing an educator today, including those teaching toxicology, is selecting what information will be taught in a course or included in a study program. This is particularly challenging when the area of study spans multiple disciplines. The field of immunotoxicology is an example of an area which requires training in multiple areas, including toxicology, pharmacology, immunology and microbiology. How these disciplines are bridged or merged together varies from institute to institute. Most of the young scientist entering the field of immunotoxicology come from one of two backgrounds. There are those which have received their primary training in the field of toxicology, either from a program which specializes in toxicology or from departments of Pharmacology and/or Toxicology. These students take additional courses in immunology and microbiology in order to focus on the immune system as their area of expertise. The other major pathway produces students with in-depth training in immunology and/or microbiology who take courses in toxicology and/or pharmacology to learn basic concepts of toxicology. Regardless of the pathway followed, a strong background in the fundamentals of toxicology is necessary when conducting immunotoxicology research. With the explosion of new information in the various fields of immunology and toxicology, the balance between training toxicologist to meet future needs and the duration of toxicological training programs is becoming a critical issue. This situation will continue to be a growing concern as we attempt to include additional training in the areas of genomics and proteomics in our toxicology programs in order to meet the toxicological needs of the future.

277 BIOENGINEERING-BRIDGING A LINK TO TOXICOLOGY.

W. E. Bentley. *Dept. of Chemical Engineering, University of Maryland, College Park, MD.* Sponsor: B. Schwetz.

According to the NIH, "Bioengineering integrates physical, chemical, or mathematical sciences and engineering principles for the study of biology, medicine, behavior, or health. It advances fundamental concepts, creates knowledge for the molecular to the organ systems levels, and develops innovative biologics, materials, processes, implants, devices, and informatics approaches for the prevention, diagnosis, and treatment of disease, for patient rehabilitation, and for improving health". This paper will highlight the integration of molecular biosciences with engineering to optimize objective functions commonly found in many engineering disciplines, specifically for the creation of new or better products and manufacturing processes. New diagnostic tools including fiber optic sensors and gene arrays, and protein synthesis systems, such as bacteria that utilize cell-to-cell communication and self-optimizing insect larvae enable new and more efficient pathways to products that are biologically based. As chemical-based products are replaced with biological-based products, so grows the interface between bioengineering and toxicology that will ensure that processes and products are developed in the context of end-use performance and safety.

278 MODELING CELL SIGNALING IN RESPONSE TO TOXICANTS.

M. A. Davis. *School of Medicine, Dept. of Pathology, University of Maryland, Baltimore, MD.*

Unlike growth factors, toxicants or pharmacological agents may utilize signaling pathways to exert adverse or desired organ responses in unique ways. The new demands in toxicology studies for cellular and molecular modeling will require that toxicologists have an appreciation for the complex series of events that occur in most cell signaling pathways. In addition, pharmacological agents are becoming more and more targeted to particular signaling proteins; therefore, new toxicologists will need to be more knowledgeable about signal transduction for proper toxicological evaluation of these targeted therapies. The response of an organ to low level toxicant exposure is often related to molecular responses elicited at the cellular level. Generation and evaluation of "new age" compounds designed for specific protein targets may likely require approaches that encompass understanding and profiling of specific molecular events that provide evidence of target specificity or potential mechanisms for observed toxicities. Indeed, the toxic effect of a chemical may be mechanistically related to its interaction with intracellular protein targets and subsequent alterations in cellular function. In this section, the potential contribution of cell signaling in mediating an organ's response to a toxicant will be discussed. Using a few specific examples, interactions between toxicants and cellular signaling proteins will be presented. These interactions will be presented in context of the cellular response and the potential to predict organ toxicity. Experimental approaches that can be used to examine specific or global effects on cellular proteins or cell signaling will also be presented. Lastly, *in vivo* models that may be appropriate for examining the relevance of a cell signaling protein to the toxicity of components will be presented. The overall goal of this section is to discuss scientific approaches that may be useful to academic discovery or regulatory scientists involved in mechanistic research or risk assessment.

279 BIOINFORMATICS-BRIDGING A LINK TO TOXICOLOGY.

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In some circles it might seem that any computer application outside of word processing and spreadsheets is "bioinformatics". A proper definition of the word, however, might be " the intersection of computer science, information technology, and biology." While classically, bioinformatics involves analysis of genetic and protein sequence data, it is often used to described statistical analysis of biological data, database construction and maintenance, database mining, and automated capture of biological data. But what does any of this have to do with toxicology? Consider that in five years or less databases WILL exist containing the mRNA and protein responses to select environmental changes (e.g. chemical treatments) for various experimental systems. But what exactly does the increase in a signal for one probe set

in a given experiment mean for human safety? How will expression responses in rats be correlated with those in dogs, monkeys or humans? How do certain transcript changes statistically correlate with chemical dose or with traditional endpoints such as clinical chemistry or histopathology. How do pathways and genes compare across species? Finally, does a given probe actually measure a given transcript, a splice variant, or even closely related sequences? These questions are in the language of toxicology, but require the tools of bioinformatics, where the sequence and computer rule, and while NDR1 and CAP43 ARE the same gene, c-src and Src are not. Toxicologists unfamiliar with these tools and concepts will be a great disadvantage in the coming decades, and the challenge of training programs is to incorporate this knowledge for both faculty and students alike.

280 THE ROLE OF THE SOCIETY OF TOXICOLOGY IN PROFESSIONAL TOXICOLOGY EDUCATION IN THE FUTURE.

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As the largest professional organization of toxicologists in the world, SOT must provide a leadership role in fostering necessary change in toxicology education. The discipline of toxicology remains highly relevant as a basic biomedical field of study, but must be able to keep pace with rapidly changing technologies and workforce demands. The Council of the SOT recently evaluated our Long Range Program and Strategic Objectives (strategic plan). Fifteen key areas of importance were 'rank ordered'. The top priority was the strategic objective to "Provide tools and resources to members that will enhance their professional and scientific development." Indeed, imbedded throughout SOT's stragetic plan is the recognition that SOT must provide direction and leadership to ensure that toxicologists of the future are properly trained in the diverse array of new tools and technologies that are changing biomedical sciences in general. Genomics, proteomics, metabolomics (and whatever the new '-omic' the future might hold!), along with computational tools of bioinformatics and the exciting possibilities of nanotechnology will become routine 'tools of the trade' in the next decade in many aspects of toxicology and risk assessment. However, it is imperative that we ensure that the fundamental concepts of toxicology are not lost in the glamour of new technologies. Although a detailed understanding of the molecular mechanism of action of a toxicant is certainly a worthwhile goal, we must ensure that the 'biology' in molecular biology is not left behind in our zeal to become more molecular. The response of the whole organism, and the many complicated interactions that occur at the 'whole animal' level, are afterall, what toxicology is all about. Through our annual meeting activities, and the new 'Current Concepts in Toxicology' focused meetings, SOT will continue to provide opportunities for all toxicologists to 'broaden their horizons' to meet the changing demands for effective product safety evaluation and risk assessment of chemical hazards.

281 DISTINCT ISOFORMS OF CALCIUM-INDEPENDENT PHOSPHOLIPASE A_2 (iPLA$_2$) ARE RESPONSIBLE FOR APOPTOSIS OF ENDOTHELIAL CELLS INDUCED BY 1-METHYLANTHRACENE (1-MA) AND PHENANTHRENE (PA).

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Smokers demonstrate a significant increase in endothelial cell (EC) injury when compared to non-smokers, a response associated with apoptosis. Specific components of cigarette smoke that induce apoptosis have not been identified. 1-MA and PA are polycyclic aromatic hydrocarbons present in high concentrations in cigarette smoke. This study was conducted to test the hypothesis that 1-MA and PA induce apoptosis of cultured human coronary artery ECs (HCAECs). HCAECs were grown to 90% confluence and treated with 30 μM 1-MA, PA or vehicle for various times. Apoptosis was detected by confocal microscopy of Annexin V/Propridium iodide staining and by Western analysis for cleavage of poly(ADP)ribose polymerase. Apoptosis was evident after 1 hour of treatment with 1-MA or PA, but not vehicle. Given that apoptosis of many cell types involves iPLA$_2$ activity, subsequent experiments were conducted to evaluate the role of PLA$_2$s in EC apoptosis. ECs were labeled for 24 h with 0.25 μCi/ml ^{3}H-arachidonic acid (AA) and release of ^{3}H-AA into the medium was used as a measure of PLA$_2$ activity. Gene expression for various iPLA$_2$ isoforms was evaluated by reverse transcription-polymerase chain reaction (RT-PCR). Both 1-MA and PA induced concentration- and time-dependent release of ^{3}H-AA that preceded the onset of apoptosis. Furthermore, the inhibitor of Group VI iPLA$_2$, bromoenol lactone (BEL), inhibited ^{3}H-AA release and apoptosis induced by 1-MA, but not PA. Conversely, the inhibitor of acidic iPLA$_2$, MJ33, significantly attenuated ^{3}H-AA release and apoptosis in response to PA, but not 1-MA. RT-PCR experiments demonstrated that the genes for these two enzymes are expressed in HCAECs. Collectively these data suggest that 1-MA and PA induce apoptosis by activating distinct isoforms of iPLA$_2$. This effect may be relevant for the mechanism by which cigarette smoking causes EC injury. (Supported by American Heart Association Grant #0160264B).

282 STATIN INDUCED APOPTOSIS IN DIFFERENTIATED RAT SKELETAL MUSCLE CELLS.

T. E. Johnson, D. R. Umbenhauer and J. F. Sina. *Merck Research Labs, West Point, PA.*

Statins are prescribed with increasing frequency due to their proven actions on treating hypercholesterolemia and their potential to lower cardiovascular disease risk . This drug class is well tolerated by most individuals, but in < 1% of the population, they cause myopathy and very rarely, rhabdomyolysis. Myopathy incidence increases with statin dose and with the coadministration of some drugs, but the mechanism is poorly understood. One fundamental question that has not been adequately addressed is how statins cause myotoxicity. Using an *in vitro* rat model of differentiated skeletal muscle, we investigated the ability of a statin to induce apoptosis in this system. L-6 myoblasts were cultured in matrigel-coated 6-well plates, in differentiation medium for six days. During this period, myoblasts formed myotubes by about Day 4 and expressed maximal levels of intracellular creatine phosphokinase, a muscle differentiation marker, by Day 6. The myotube cultures were then treated with Compound A (lactone form), Compound B (open acid form of A) or Compound C (epimer, inactive against HMG-CoA reductase). Apoptosis was assessed over a timecourse by TUNEL and by activated caspase-3 staining. The lactone (10 μM) caused a time-related increase in TUNEL positive nuclei, from 3 to 8 fold over control at 7 and 24 hr, respectively. Caspase-3 activation was induced by about 3 fold at 3 hr and increased to about 8 fold at 24 hr. Apoptosis was detected at minimum doses of 1 μM at 7 hr and 50 nM at 72 hr. Similar results were seen with the open acid. In contrast, the epimer, at a 100 fold higher concentration, induced a 3 fold increase in apoptosis, only after 48 hr. The statin induced-apoptotic response could be inhibited by 50 μM mevalonate and by a specific caspase 3 inhibitor (Z-DEVD-FMK). Taken together, these results suggest that statins induce apoptosis in differentiated rat skeletal muscle cultures through a mechanism that involves inhibition of HMG-CoA reductase and activation of caspase-3. Until substantiated, these *in vitro* studies might not reflect the process that occurs *in vivo* (animals or humans).

283 ROLE OF FAS-FASL INTERACTIONS IN ESTRADIOL-INDUCED THYMIC ATROPHY AND APOPTOSIS.

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Previous studies from our laboratory have demonstrated that ©¬-estradiol-17-valerate (E2) induces apoptosis in thymocytes leading to thymic atrophy. In the current study we investigated the role of Fas-FasL interactions in E2-induced thymic atrophy and susceptibility to apoptosis using C57BL/6 wild-type (+/+), Fas-deficient (C57BL/6-*lpr/lpr*) and FasL-deficient (C57BL/6-*gld/gld*) mice. The +/+/, *lpr* and *gld* mice were treated with various concentration of E2 such as 75, 25, 5, 1, or 0.1mg/kg body weight or vehicle. The thymi from these mice were harvested on days 1, 4, and 7 following treatment and cellularity and apoptosis were determined. Treatment with E2 caused a decrease in thymic cellularity at all doses except 0.1mg/kg, in all three groups of mice, particularly on days 4 and 7. Interestingly, however, the degree of thymic atrophy in *lpr* and *gld* mice was significantly less than that seen in wild-type mice. When thymocytes were analyzed for apoptosis, cells from *lpr* and *gld* mice showed decreased levels of apoptosis. Moreover, cDNAarray analysis of gene expression revealed that treatment with E2 upregulated several genes involved in apoptosis including FasL, caspases, and TRAIL. At higher doses, E2 caused a decrease in cellularity of the spleen in wild-type mice. Also, E2 treatment decreased the proliferative response of T cell to the superantigen, SEA. Together, the current study suggests that estrogen may cause immunosuppression in activated T cells by inducing FasL gene and triggering apoptosis through paracrine or autocrine pathways (supported in part by NIH grants- ES 09098, HL 58641 and AI 01392).

284 DIETHYLSTILBESTROL INDUCES APOPTOSIS IN LYMPHOMAS AND LEUKEMIAS: ROLE OF FAS LIGAND UPREGULATION.

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Endocrine disrupting compounds like estrogens have the ability to alter the immune system. One such compound is a synthetic nonsteroidal estrogen, diethylstilbestrol (DES). Studies from our Lab. have demonstrated that estrogen induces

thymic atrophy by triggering apoptosis. In the current study, we investigated the ability of DES to induce apoptosis *in vitro* in murine lymphomas, LSA and EL-4 as well as human lymphomas and leukemias, Jurkat, Sup T1, HUT 78 and Molt-4. For this purpose, tumor cells were cultured with DES (50, 25, 10, 1 microM) in serum-free medium. Twenty four hours later, the cell numbers were enumerated by trypan blue dye exclusion and apoptosis was detected by TUNEL staining. Studies demonstrated that DES at concentrations of 10-50microM induced varying levels of apoptosis in all the cell lines tested. Inasmuch as Fas-Fas ligand (FasL) interactions play a crucial role in induction of apoptosis, further studies were performed to assess the level of expression of these molecules on the cells following treatment with DES. It was observed that while Fas expression was not altered, there was significant upregulation of FasL. The induction of apoptosis by DES was inhibited by treatment with caspase inhibitors including z-VAD-fmk, z-WEHD-fmk, z-VDVAD-fmk and z-IETD-fmk. Together, these studies demonstrated that DES-induced apoptosis of tumor cells may involve the death-receptor pathway (Supported in part by grants from NIH: ES09098 and HL58641).

285 LIGATION OF CANNABINOID RECEPTORS LEADS TO INDUCTION OF APOPTOSIS IN TRANSFORMED IMMUNE CELLS.

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Exposure to delta 9-tetrahydrocannabinol (THC), an active component of marijuana can lead to suppression of the immune response although the mechanism remains unclear. In the current study, we tested the hypothesis that cannabinoids induce toxicity in lymphoid cells by triggering apoptosis. To this end, we screened a large number of murine and human transformed cells of immune origin for the expression of cannabinoid receptors and their susceptibility to apoptosis induced by THC and other cannabinoid receptor agonists. RT-PCR and Western blot analysis revealed that murine tumor cell lines EL-4, LSA, and P815 expressed both the CB1 and CB2 receptors. Exposure of EL-4, LSA, and P815 cells to THC led to a significant reduction in cell viability and increase in apoptosis. Exposure of EL-4 tumor cells to the synthetic cannabinoid, HU-210 also led to significant induction of apoptosis, while exposure to WIN55212 was less effective. Treatment with THC *in vivo* led to a significant reduction in the number of viable tumor cells and an increase in tumor cell apoptosis. Examination of a number of human leukemias and lymphomas revealed that unlike the murine tumors, Jurkat, Molt-4, and Sup-T1 cells express only CB2 receptors. Exposure of these tumor cells to 5 μM THC or HU-210 led to significant induction of apoptosis. The induction of apoptosis by THC in tumor cells was mediated through the cannabinoid receptors inasmuch as it was inhibited in the presence of CB1 and/or CB2 receptor antagonists, SR14176 and SR144528. Together, these data demonstrate that ligation of cannabinoid receptors can induce apoptosis in transformed immune cells. Thus, cannabinoids may induce immunotoxicity by triggering apoptosis in lymphoid cells through cannabinoid receptors. These studies also suggest that cannabinoid receptor agonists may serve as anticancer agents (Supported in part by grants NIH DA14885, ES09098 and HL58641)

286 DIFFERENTIAL INVOLVEMENT OF THE MITOCHONDRIAL/CASPASE-9-DEPENDENT APOPTOTIC PATHWAY IN HYDROQUINONE TREATED HL-60 AND JURKAT LEUKEMIA CELLS.

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Hydroquinone (HQ), a benzene metabolite induces apoptosis in hematopoietic cells and is believed to be involved in the pathophysiology of benzene-induced aplastic anemia. Here, we investigated the involvement of mitochondria and activation of the apical caspase-9 in HQ induced apoptosis in myeloperoxidase (MPO)-rich HL-60 and MPO-deficient Jurkat T cell. Treatment of HL-60 and Jurkat cells with HQ (75μM and 50μM respectively) resulted in comparable apoptosis as assessed by flow cytometric and biochemical changes, including phosphatidylserine (PS) exposure, loss of mitochondrial membrane potential (MMP) and caspase-9 cleavage. In HL-60 cells, pretreatment with the pan-caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (ZVAD) did not inhibit PS exposure and the loss of MMP was relatively unaffected. Interestingly, activation of caspase-9 was only inhibited to the p37/35kDa subunits without any intact protein observed. In marked contrast, in HQ-treated Jurkat cells, ZVAD significantly abrogated PS exposure and loss of MMP with caspase-9 remained in its zymogen form. These data suggest that in HQ-treated HL-60 cells, the event(s) leading to mitochondrial alteration occurs *via* a ZVAD insensitive mechanism suggesting for a caspase-independent pathway in HQ treated HL-60 cells. In contrast, in HQ-treated Jurkat cells, the event(s) leading to the participation of mitochondria/caspase-9 occurs in a ZVAD sensitive manner indicating a caspase-dependent process in this apoptotic death. These differential effects may be attributed to MPO-catalyzed activation of HQ to highly reactive quinones and reactive oxygen species in HL-60 cells, and, as a result, specific death pathway is recruited. (Supported in part by ES 09554)

287 MITOCHONDRIAL SUPEROXIDE DISMUTASE INHIBITS MITOCHONDRIAL OXIDATIVE STRESS AND APOPTOSIS IN CULTURED RAT HEPATOCYTES AFTER TUMOR NECROSIS FACTOR-α (TNFα).

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Onset of the mitochondrial permeability transition (MPT) is a key event signaling TNFα-induced apoptosis in cultured rat hepatocytes. Previously, we showed that pre-exposure to non-toxic oxidative stress sensitizes hepatocytes to the TNFα-induced MPT and apoptosis. In the current study, we tested whether expression of cytosolic and mitochondrial superoxide dismutase (SOD1 and SOD2, respectively) inhibits the MPT and apoptosis promoted by TNFα plus oxidative stress. Rat hepatocytes were infected with an adenovirus (Ad5IkB, 30pfu) for 2 hours to induce I?B superrepressor. Some hepatocytes were also infected with adenoviruses (30 pfu) expressing SOD1 or SOD2 for 3 hours. Afterwards, the hepatocytes were exposed to 1 μM t-butylhydroperoxide (t-BuOOH), and TNFα (30 ng/ml) was added 2 h later. Cell viability and cellular oxidative stress were determined by propidium iodide (PI) and dichlorodihydrofluorescein (DCF) fluorometry, respectively. Mitochondrial reactive oxygen species (ROS) formation (DCF), mitochondrial membrane potential (tetramethylrhodamine methylester) and mitochondrial inner membrane permeabilization (DCF leakage) were monitored by confocal microscopy. In t-BuOOH pretreated cells, TNFα accelerated ROS formation, as indicated by a 387% increase of DCF fluorescence over 3h. In hepatocytes expressing SOD2, ROS was inhibited to 182%. SOD2 also inhibited TNFα-induced cell killing from 68% after 12 h to 25%. Expression of SOD1 did not protect against cell killing. Confocal microscopy showed increased mitochondrial ROS generation, mitochondrial depolarization and mitochondrial membrane permeabilization, which began 4 h after TNFα plus t-BuOOH. SOD2 expression suppressed mitochondrial ROS, depolarization and permeabilization even after 8 h. In Conclusion, mitochondrial superoxide generation sensitizes cultured rat hepatocytes to TNFα-induced apoptosis by promoting onset of the MPT. Expression of mitochondrial but not cytosolic SOD blocks this apoptotic signaling.

288 PEROAXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA ENHANCES SUSCEPTIBILITY TO APOPTOSIS INDUCERS IN MOUSE LIVER.

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Peroxisome proliferators (PP) are a large class of structurally dissimilar chemicals that induce hepatocellular proliferation and peroxisome proliferation through the nuclear receptor family member peroxisome proliferator-activated receptor alpha ($PPAR_\alpha$). We performed gene expression profiling to examine differences in gene expression between untreated wild-type and $PPAR_\alpha$-null mice. $PPAR_\alpha$-null mice exhibited altered expression of a number of genes that play roles in apoptosis, favoring decreased susceptibility to apoptosis. We hypothesized that $PPAR_\alpha$ activation positively influences induction of apoptosis in the liver. Wild-type and $PPAR_\alpha$-null mice were treated in separate experiments with two apoptosis inducers: Jo-2 antibody, which induces the Fas-dependent death pathway, and conconavalin A (ConA), which induces apoptosis through an unknown pathway. Mice were pretreated with and without the PP, WY-14, 643 (WY). Wild-type mice treated with WY were more sensitive to induction of apoptosis by Jo2 antibody than untreated wild-type mice or untreated or WY-treated $PPAR_\alpha$-null mice. After exposure to Jo2, morbidity and mortality were significantly higher in the presence of WY in wild-type mice and paralleled increases in hepatocyte apoptosis and necrosis. Wild-type mice treated with WY were also more sensitive to the apoptotic effects of ConA. We examined the expression of a number of gene products in the liver that influence apoptosis including those found to be altered by gene expression profiling. In the absence of apoptosis-inducer treatment, wild-type mice treated with WY exhibited increases in the activated form of caspase-9 which paralleled the decrease in the anti-apoptotic protein Mcl-1, a member of the Bcl-2 family. These data demonstrate that $PPAR_\alpha$ activation can significantly enhance the sensitivity of apoptosis inducers in the mouse liver possibly through alteration in the expression of proteins that regulate apoptosis.

289 S-(1, 2-DICHLOROVINYL)-L-CYSTEINE (DCVC)-INDUCED CHANGES IN EXPRESSION OF GENES RELATED TO APOPTOSIS AND CELL GROWTH IN HUMAN PROXIMAL TUBULAR CELLS.

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DCVC, the cysteine conjugate of trichloroethylene, is a potent nephrotoxicant. Using a broad range of concentrations, including environmentally relevant ones (10 to 500 μM), we showed that DCVC induces necrosis, apoptosis and cell proliferation in a time- and concentration-dependent manner in primary cultures of human proximal tubular (hPT) cells. Significant apoptosis was observed with as low as 10 μM DCVC at 1 hr, using cell cycle analysis with propidium iodide staining or annexin V staining and flow cytometry. Maximal apoptosis occurred with 50 μM DCVC at 4 hr incubation. Similar time course was observed for enhanced cell proliferation, as assessed by cell cycle analysis with flow cytometry and incorporation of radiolabeled dTTP. In contrast, significant increases in necrosis (as assessed qualitatively by morphology and quantitatively by release of lactate dehydrogenase) did not occur until later incubation times (≥ 24 hr) and higher DCVC concentrations (≥ 100-200 μM). To gain more insight into the regulation of these responses, we measured protein expression of several genes that are related to apoptosis and cell growth regulation. Primary cultures of hPT cells were incubated with 10, 50, 100, 200, or 500 μM DCVC for 0.5, 1, 2, 4, 8, or 24 hr and protein expression was determined by Western blot analyses. Expression of several proteins related to apoptosis were altered, including αBax (decrease), Apaf1, Bcl2 and caspase-9 (all increase), and αPARP (increase in low molecular weight fragments). cJun increased only at low DCVC concentrations, whereas expression of Hsp27, NF-κB p65 subunit, and P53 all showed modest to large increases that were clearly dependent on DCVC concentration and exposure time. These results are consistent with the prominence of apoptosis at low concentrations of DCVC and with a role for oxidative stress and changes in regulation of cell growth in the response of hPT cells that are exposed to DCVC. (Supported by NIEHS Grant ES08828.)

290 ACTIVATION OF THE RAS/MAPK PATHWAY PROTECTS HYPOXIC TUMOR CELLS AGAINST P53-DEPENDENT APOPTOSIS.

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Tumor hypoxia develops in most solid tumors because of inadequate vasculature or abnormal vascular architecture. Frequently, these hypoxic cancer cells resist radiation and chemotherapy. The aim of this study was to characterize the roles of p53 and the Ras/MAPK pathway in apoptosis to hypoxic tumor cells. p53+/+ and -/- mouse embryonic fibroblasts (MEF) transformed with proto-oncogenes Ras and E1A were exposed to hypoxia for 7 h. Hypoxia caused 29 ± 4.2% and 4 ± 1.2% apoptosis in p53+/+ and -/- MEF, respectively, as assessed by changes in nuclear morphology. Hypoxia had no effect on p53+/+ and -/- MEF without Ras and E1A. Surprisingly, the specific inhibitor of mitogen-activated protein kinase kinase (MKK1), PD98059 (50 μM), accelerated hypoxia-mediated apoptosis (85 ± 4.6%) in p53+/+ but not p53-/- MEF. PD98059 alone caused 45 ± 8.8% apoptosis in p53+/+ MEF. To investigate whether apoptosis induced by inhibition of MKK1 was mediated by activation of procaspase-8, we measured caspase-8 activation by fluorogenic assay and Western blot. Both methods showed that 4 h exposure to hypoxia or PD98059 alone did not activate caspase-8. However, the combination of hypoxia and PD98059 caused a 2-fold increase of caspase-8 activity in p53+/+ MEF but not in p53-/- MEF. To determine whether caspase-8 activation caused translocation of Bid from the cytosol to mitochondria, we transiently transfected p53+/+ MEF with cDNA Bid-GFP. Before hypoxia the pattern of Bid-GFP fluorescence was diffuse indicating a cytosolic localization. After hypoxia Bid-GFP fluorescence became punctate in the presence of PD89059 and colocalized with MitoTracker Red. Collectively, our results indicate that p53 sensitizes MEF to hypoxic apoptosis. Since inhibition of the Ras/MAPK pathway sensitizes hypoxic cancer cells to caspase-8-activation and apoptosis, we conclude that the Ras/MAPK pathway provides a survival signal against p53-mediated hypoxic apoptosis. MAPK inhibitors may therefore be useful as an adjunct chemotherapy for hypoxic solid tumors. Supported by NS39469.

291 ALTERED APOPTOTIC RESPONSE IN 4-HYDROXYEQUILENIN-TREATED MCF-10A CELLS.

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Excessive exposure to synthetic and endogenous estrogens has been associated with the development of cancer in several tissues including the breast. 4-Hydroxyequilenin (4-OHEN), a major metabolite of equine estrogens present in Premarin® estrogen replacement formulation, has been shown to induce DNA damage and apoptosis in human breast cancer cells. It also has the potential to be a tumor promoter and complete carcinogen *in vitro*. To further understand the mechanism(s) of estrogen carcinogenesis, we have used human non-tumorigenic breast epithelial MCF-10A cells to study the toxic effects of 4-OHEN. We observed cytotoxicity in MCF-10A cells induced by 4-OHEN; LC_{50} for 18 h-treatment was 10.7 ± 1.9 μM. 4-OHEN also caused dose-dependent increases in apoptosis and DNA damage as measured by the DAPI nuclear screening assay and the Comet assay, respectively. In the present study, we found that treatment with 100 nM 4-OHEN biweekly for 2 weeks induced cellular transformation in these human breast epithelial cells. Interestingly, after the treatment of cells with 100 nM 4-OHEN biweekly for 4 weeks, the treated MCF-10A cells were resistant to 4-OHEN- or cisplatin-induced apoptosis. The mechanism of cellular resistance to apoptosis is currently under investigation. Our data indicated that long-term low-level estrogen exposure could induce DNA damage and initiate cells that become resistant to apoptosis, which could contribute to the mechanism(s) of equine estrogen carcinogenesis. (Supported by NIH grant CA73638).

292 THE SEROTONERGIC NEUROTOXICITY OF α-METHYLDOPAMINE THIOETHERS REQUIRES THE SEROTONIN TRANSPORTER-DEPENDENT GENERATION OF REACTIVE OXYGEN SPECIES.

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We have previously shown that thioether metabolites of 3, 4-methylenedioxyamphetamine (MDA) and 3, 4-methylenedioxymethamphetamine (MDMA, ecstasy), 5-(glutathion-S-yl)-α-methyldopamine (5-[GSyl]-α-MeDA) and 2, 5-*bis*(GSyl)-α-MeDA, are selective serotonergic neurotoxicants. Using primary cell cultures derived from the striatum (ST) and hippocampus (HP), and the serotonergic JAR cell line, we examined the mechanism(s) involved in α-MeDA-thioether-mediated toxicity. Immunohistochemical staining indicated that both 5-(GSyl)-α-MeDA and 2, 5-*bis*(GSyl)-α-MeDA produced a significant loss in serotonin (5-HT) positive cells in HP, ST, and JAR cell cultures. HP cell cultures, which contain a higher proportion of 5-HT-positive cells, were more sensitive to toxicity than primary cultures of ST cells. TUNEL staining indicated that cell death in all three models was apoptotic. Although the 5-HT transport inhibitor, fluoxetine (FX), attenuated 5-(GSyl)-α-MeDA and 2, 5-*bis*(GSyl)-α-MeDA mediated cell loss, the dopamine transport inhibitor, nomifensine, had no effect on cell death. Subsequent uptake experiments revealed 5-(GSyl)-α-MeDA and 2, 5-*bis*(GSyl)-α-MeDA significantly inhibited 5-HT uptake in HP, ST, and JAR cells. This effect was potentiated with FX. Interestingly, in HP cells, and to some extent, in JAR cells, 5-(GSyl)-α-MeDA and 2, 5-*bis*(GSyl)-α-MeDA *increased* dopamine uptake, an effect that was blocked by FX. Finally, in all cell types, 5-(GSyl)-α-MeDA and 2, 5-*bis*(GSyl)-α-MeDA induced a significant increase in the generation of reactive oxygen species (ROS). Pretreatment with FX attenuated ROS production while nomifensine had little effect, suggesting that the 5-HT transporter is important for the production of ROS. These results are consistent with the view that MDA and MDMA induced serotonergic neurotoxicity are mediated *via* the formation of thioether conjugates capable of generating ROS in a manner dependent upon the 5-HT transporter.

293 TRICHLOROETHYLENE (TCE) ELICITS TOXICITY IN THE MALE REPRODUCTIVE SYSTEM.

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We have tested the hypothesis that TCE is metabolized by CYP2E1 and elicits toxicity in the male reproductive system. We have exposed mice to TCE by inhalation (1000 ppm) for 6 h per day for 5 days per week for a total of 1 to 4 weeks. Internal exposure to TCE was monitored by measuring urinary levels of the TCE metabolites, trichloroacetic acid (TCA) and trichloroethanol (TCOH). We have determined CYP2E1 expression in epididymis and testis by i) immunohistochemical staining, ii) immunoblotting, and iii) measurement of p-nitrophenol hydroxylase activity. We have also determined formation of chloral in incubations of microsomes from epididymis and testis and in microsomes preincubated with a CYP2E1 antibody. Our results showed that urinary levels of TCA and TCOH increased between the first and second week, and remained elevated thereafter. CYP2E1 is localized in epithelial cells of the epididymis and Leydig cells of the testis. Immunoblots of CYP2E1 showed that the protein was present at higher levels in epididymis than in testis. This finding coincided with the 2-fold higher level of hydroxylase activity detected in epididymis *vs.* testis. Formation of chloral in incubations of microsomes from epididymis and testis was time- and concentration-dependent. Peak levels were detected at 60 min of incubation time at a concentration

of 3.0 mM of TCE. Chloral formation by microsomes from epididymis was significantly higher than by those from the testis. Levels were significantly inhibited in epididymal microsomes preincubated with a CYP2E1 antibody. These metabolic parameters correlated with structural changes that were most evident in the epididymal epithelial cells of mice exposed to TCE by inhalation. These results showed that TCE metabolism in the male reproductive system leads to adverse consequences that are manifested to the greatest extent in the epididymis. Supported by Health Canada/Environment Canada and NIEHS RO1-ES08828.

294 FORMATION OF FREE RADICALS FROM METABOLISM OF 1, 1-DICHLOROETHYLENE (DCE) IN MURINE AND HUMAN LUNG AND LIVER.

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DCE produces lung and liver toxicity upon exposure. The toxic response has been ascribed to formation of reactive intermediates from CYP2E1-mediated metabolism of DCE. Here, we have tested the hypothesis that, in addition to formation of reactive metabolites, free radicals are also produced by DCE exposure. We have used a microsomal incubation system and α-phenyl-*t*-butylnitrone (PBN) as a trapping agent. The PBN spin adducts were detected by electron paramagnetic resonance spectroscopy. Free radicals generated through DCE metabolism were determined in incubations of lung and liver microsomes from mice and humans. The role of CYP2E1 in free radical production was investigated in incubations containing CYP2E1-expressed β-lymphoblastoid microsomes. The potential of glutathione (GSH) to inhibit free radical formation was determined in microsomal incubations supplemented with GSH. Microsomes were incubated with DCE (2-4 mM) in the presence of NADPH and PBN. The PBN adducts were extracted with benzene and subjected to EPR analysis. Free radicals were formed from DCE in incubations containing NADPH, and were not detected in the absence of NADPH. Their formation in lung and liver microsomal incubations was time- and dose-dependent. The amounts of free radicals generated by human microsomes were similar to those in mice. Free radicals were also detected in incubations containing CYP2E1-expressed lymphoblastoid microsomes. Levels of the radicals were decreased in incubations supplemented with GSH. Analysis of hyperfine splitting constants indicated formation of an oxygen-centered radical. These results showed that free radicals are formed from P450-mediated metabolism of DCE, and implicates CYP2E1 as being involved. Supported by Grant MOP 11706 from Canadian Institutes of Health Research.

295 RELATIONSHIP OF THE SINGLE-ELECTRON REDUCTION POTENTIAL OF QUINONES TO THEIR ABILITY TO UNDERGO REDOX CYCLING MEDIATED BY RAINBOW TROUT MICROSOMAL FLAVOPROTEINS.

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Exposure to quinones can result in cytotoxicity due to electrophilic arylation or redox cycling *via* enzymatic reduction to the semiquinone radical with subsequent transfer of an electron to molecular oxygen to form superoxide anion radical ($O_2^{\cdot-}$)and subsequent regeneration of the parent quinone. To assess the relationship between the redox cycling ability of quinones in rainbow trout and their single-electron reduction potentials (E^1), the production of $O_2^{\cdot-}$ by a series of quinones with potentials ranging from -348 to +671 mV was determined using two assays. Rainbow trout liver microsomes, containing NADPH cytochrome P450 reductase and NADH-cytochrome b5 reductase, were exposed to quinones in the presence of NADH or NADPH. Generation of $O_2^{\cdot-}$ was measured as O_2 consumption and acetylated cytochrome c reduction. The reduction of acetylated cytochrome c is a more sensitive indicator of $O_2^{\cdot-}$ production. The peak and lower limit for acetylated cytochrome c reduction were E^1 of-36mV and -348mV with NADPH cofactor and E^1 of -203mV and -348mV with NADH cofactor. A narrower range of redox activity was observed with the O_2 consumption assay. The peak rate for oxygen consumption was observed at the same E^1 as for the acetylated cytochrome c assay and the lower limit for reduction was E^1 of -348mV with both NADPH and NADH. These redox windows for rainbow trout microsomes are comparable to those reported for NADPH cytochrome P450 reductase and NADH-cytochrome b5 reductase in rat hepatocytes. In both rats and rainbow trout the peak reduction occurs at a more positive E^1 with NADH cofactor as compared to that with NADPH cofactor. In contrast to rat flavoproteins, the peak rate of quinone redox cycling facilitated by rainbow trout flavoproteins occurred with NADH as the cofactor rather than NADPH. This abstract does not necessarily reflect EPA policy.

296 MASS SPECTRAL CHARACTERIZATION OF DICHLOROACETIC ACID-MODIFIED GLUTATHIONE TRANSFERASE ZETA.

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Glutathione transferase zeta (GSTZ) is broadly distributed in eukaryotic species. Four polymorphic variants of hGSTZ1-1 have been described. GSTZ is identical with maleylacetoacetate isomerase, which catalyzes the cis-trans isomerization of maleylacetoacetate. GSTZ also catalyzes the biotransformation of a range of α-haloalkanoic acids, including dichloroacetic acid (DCA), which inactivates and covalently modifies the enzyme. The objective of the research was to determine the partition ratio for the inactivation of hGSTZ1a-1a and -1c-1c, the stoichiometry of [1-^{14}C]DCA binding to hGSTZ1c-1c, and the labeling of C16A hGSTZ1c-1c by [1-^{14}C]DCA and to characterize DCA-inactivated hGSTZ1c-1c by MALDI-TOF MS and ESI/MS/MS. The partition ratios for the DCA-induced inactivation of hGSTZ1a-1a and -1c-1c were 2.23 ± 0.27 x 10^3 and 5.7 ± 0.5 x 10^2, respectively. hGSTZ1c-1c incorporated an estimated 0.45 mol [1-^{14}C]DCA/mol enzyme. C16A hGSTZ1c-1c was resistant to inactivation by DCA and incorporated less [1-^{14}C]DCA than hGSTZ1c-1c: 11% incorporation was observed relative to hGSTZ1c-1c. MALDI-TOF MS and ESI/MS/MS characterization of DCA-inactivated hGSTZ1c-1c revealed 2 adducts on the active site-containing tryptic peptide (14)SSCSWR(19) (M+H$^+$ *m/z* 725.4). One modified peptide (M+H$^+$ *m/z* 1030.4) was characterized as the peptide/GSH mixed disulfide. The other modified peptide (M+H$^+$ *m/z* 1088.4) contained both GSH and the carbon skeleton of DCA, presumably in a dithioacetal linkage. These data support a mechanism of inactivation whereby GSH displaces the first chlorine atom of DCA and cysteine-16 in the enzyme active site displaces the second to result in a covalently modified and inactivated enzyme. (Supported by ES03127, ES10056, and ES06694).

297 1, 1-DICHLOROETHYLENE (DCE) ELICITS MITOCHONDRIAL DYSFUNCTION IN MURINE LIVER.

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Hepatotoxicity induced by DCE, a chemical used in the manufacture of plastics, is ascribed to cytochrome P450-dependent metabolism to reactive intermediates. We have tested the hypothesis that mitochondria are a primary target of toxicity by investigating dose- and time-dependent effects of DCE on mitochondrial respiration. We have also determined the effectiveness of *N*-acetyl-L-cysteine (NAC) to increase glutathione levels and to protect against or decrease potential mitochondrial respiratory perturbations. Liver mitochondria were isolated 2 h after DCE (50, 75, 100, 125, and 150 mg/kg) treatment. Glutamate (complex 1)- and succinate (complex 2)-supported mitochondrial respiration was assessed by measurement of state 3 (ADP-stimulated) and state 4 (resting) rates of oxygen consumption. The corresponding respiratory control ratios (RCR: state 3/state 4) and ADP:O ratios were then calculated. A DCE dose of 125 mg/kg significantly inhibited state 3 respiration at complex I and II. This inhibition led to a significant reduction in corresponding RCRs and ADP:O ratios. Respiration at complex I and II was completely uncoupled with doses of 150 mg/kg or higher. State 3 respiration rates and RCRs for glutamate-supported respiration were significantly decreased at 20, 30, 60, 90, and 120 min after DCE (125 mg/kg) treatment, while those for succinate-supported respiration were significantly decreased at 90 and 120 min. ADP:O ratios for glutamate-supported respiration were significantly decreased at 60, 90, and 120 min, whereas those for succinate-supported respiration were significantly decreased at 120 min after DCE exposure. Pretreatment with NAC (1200 mg/kg) reduced the DCE-induced impairment of mitochondrial respiration, suggesting a role for glutathione depletion in the development of toxicity. These results indicated that DCE exposure produces mitochondrial dysfunction in murine liver. (Supported by Grant MOP 11706 from the Canadian Institutes of Health Research).

298 ALTERATION OF THE RETINOBLASTOMA PATHWAY IN BENZENE-INDUCED THYMIC LYMPHOMAS FROM P53+/- MICE.

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Exposure of C57BL/6-Trp53 haploinsufficient (N5) mice (p53+/- mice) to inhaled benzene induces a high frequency of thymic lymphomas. Loss of p53 function is a common feature of human cancer and we have previously shown that loss of the

functional p53 allele was a common feature of the benzene-induced tumors supporting the use of these mice to examine the molecular mechanisms of cancer. We then examined the benzene-induced tumors for altered expression of genes involved in the retinoblastoma (Rb) pathway since this pathway is also commonly altered in human tumorigenesis. Real-time quantitative RT-PCR and Western blot analysis were used to determine the levels of genes involved in the Rb pathway (p15INK4b, p16INK4a, p19ARF, Rb, cyclin D1, cyclin D2, and E2F-1) in 18 benzene-induced thymic lymphomas from p53+/- mice. Virtually all genes showed decreased mRNA levels in the tumors, however all tumors showed increased levels of p16INK4a mRNA and protein compared to normal thymus, suggesting that increased levels of p16INK4a mRNA can be used as a biomarker for benzene-induced transformation. Bisulfite DNA sequencing showed that the tumors exhibited reduced methylation at specific CpG sites within the promoter region of p16INK4a suggesting that altered methylation contributed to the p16INK4a mRNA increases. Our data show that benzene-induced thymic lymphomas from p53+/- mice exhibited alterations common to certain human cancers, providing further support for the use of these mice as a model to examine the mechanisms involved in human carcinogenesis.

299 PROGRESSIVE ALTERATIONS IN DNA METHYLATION DURING TUMORIGENESIS.

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DNA methylation (DNA-Me), 5-methylcytosine content of DNA, is an epigenetic process involved in transcriptional control. Typically, there is an inverse relationship between the level of DNA-Me and the degree of transcription. Multiple alterations in DNA-Me occur in precancerous and cancerous tissue, e.g., human colon cancer, suggesting that aberrant patterns of DNA-Me may be a cause of the abnormal transcription of genes involved in carcinogenesis. DNA-Me patterns in tumor tissue are often characterized by a decrease in overall, or global, methylation with a concurrent increase at specific GC-rich regions, e.g., CpG islands, in the 5'-flanking, promoter region of genes. Increased DNA-Me may silence tumor suppressor genes while decreases may facilitate expression of oncogenes. In this study, we employ a two-stage, initiation/promotion, skin tumor model of carcinogenesis. SENCAR mice were initiated with 75 mg dimethylbenz(a)anthracene and promoted with 27 mg of cigarette smoke condensate administered thrice weekly for 29 wk. DNA-Me status was assessed using both an *SssI* methylase assay and an arbitrarily primed PCR procedure to measure global and GC-rich region methylation, respectively. Methylation in GC-rich regions in both tumors and promoted non-tumor skin tissue increased as compared to controls. However, global methylation was decreased only in tumor tissue. Thus, we conclude that an increase in methylation at GC-rich regions may be an early event that occurs in initiated cells that proliferate in response to promoter-treatment. With continued promotion, a subpopulation of these cells that exhibit a global decrease in methylation, while maintaining selected regions of hypermethylation, expand clonally and progress to tumors. The skin tumor initiation/promotion model offers the potential to discern the role that particular alterations in DNA-Me play at specific stages of carcinogenesis. Additionally, methylation status may serve as a useful endpoint in the safety assessment of tumor promoters.

300 EFFECT OF ENVIRONMENTAL CARCINOGENS ON THE METHYLATION OF IGF-II AND C-MYC GENES IN RODENTS.

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Dichloroacetic acid (DCA), trichloroacetic acid (TCA), dibromoacetic acid (DBA), bromodichloromethane (BDCM), methylene chloride (MC) and arsenic (As) are non-genotoxic carcinogens in various organs. DNA methylation as 5-methylcytosine (5-MeC) is a mechanism to regulate the transcription of mRNA. Hypomethylation of DNA and of genes including insulin-like growth factor 2 (IGF-II) and the c-myc protooncogene has been proposed to be an epigenetic mechanism for non-genotoxic carcinogens. We determined the effect of DCA, TCA, DBA, BDCM, MC and As on methylation in the promoter of the c-myc gene and in the differential methylation region 2 of the IGF-II gene. B6C3F1 mice were administered in their drinking water 3.2 mg/l DCA or 4.0 mg/l TCA for 24-96 hours and for 28 days or were administered DBA (1000 and 2000 mg/l) and DBCM (350 and 700 mg/l) for 2-28 days. Methylene chloride was administered to mice by inhalation at 1.04, 3.48 and 10.44mg/liter for 5 days. Mice received by gavage dimethylarsinic acid (DMA) at 600 mg/kg for 5 days. Fisher 344 rats were administered for 2-28 days, DBA (1000 or 2000 mg/l) and BDCM (350 or 700 mg/l) in drinking water or were administered BDCM (50 and 100 mg/kg) by gavage. Methylation of the two genes was determined using the bisulphite-modified DNA sequencing procedure. Methylation in the IGF-II and c-myc genes was decreased in the liver and kidney of BDCM, DMA and MC-treated mice, in the kidney of BDCM and DMA-treated mice, in the skin of DMA-treated mice, in the bladder with DCA, TCA and DBA-treated mice, in the lung of MC-treated mice and in the liver of BDCM and DBA-treated rats. Hypomethylation of the genes was observed after exposure of at least 96 hours and was maintained for 28 days. These findings suggested that hypomethylation of DNA and of genes is an epigenetic activity of non-genotoxic carcinogens so that it could be involved in their mechanism

301 HYPOMETHYLATION OF GENES IN CHEMICAL CARCINOGEN-INDUCED RODENT TUMORS.

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DNA methylation at the 5-position of cytosine in CpG dinucleotides (CpG sites) or CpNpG trinucleotides (CpNpG sites) appears to play an important role in regulating gene expression. Many human and some animal tumors have been reported to have decreased methylation (hypomethylation) of DNA and of different genes. This includes our previous demonstration that mouse liver tumors initiated by MNU and promoted by either DCA or TCA contained hypomethylation of DNA and of the c-myc gene. The methylation of two protooncogenes, c-myc and IGF-II were chosen as indicators of the methylation status of tumor DNA. Methylation of the c-myc gene was determined by Hpa II-digestion followed by Southern blot analysis and methylation of the IGF-II gene was determined by the bisulfite-treated DNA sequencing procedure. Lung tumors were induced in strain A mice by vinyl carbamate and benzo(a)pyrene [B(a)P] and colon tumors were induced in C3H mice and F344 rats by azoxymethane (AOM). In colon, liver, and lung tissue from untreated mice, 21-24 of the 28 CpG sites (75-86%) and none of the three CpNpG sites were methylated in the probed region of the IGF-II gene. In DCA- and TCA-promoted liver tumors, in vinyl carbamate and B(a)P-induced lung tumors and in AOM-induced colon tumors the level of methylation in this region of IGF-II gene was reduced to less than 25% of the CpG sites. Similar hypomethylation of the c-myc gene was also found in these tumors. These findings support the suggestion that hypomethylation of DNA and of genes is a common molecular event in rodent tumors. Furthermore, since we have found that short-term treatment with non-genotoxic carcinogens induced similar hypomethylation of genes prior to the occurrence of tumors it is proposed that hypomethylation of DNA is involved in the activity of these carcinogens.

302 EXPRESSION OF THE ARYL HYDROCARBON (AH) RECEPTOR AS A MARKER OF HUMAN BREAST CANCER PROGRESSION.

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The Ah receptor (AhR), a ligand-activated helix loop helix transcription factor, binds environmental polyaromatic hydrocarbons (PAH) such as dioxin, and mediates their toxic effects. This study was designed to investigate the expression of AhR and its possible role in breast carcinogenesis, using the Sager's 21T human breast carcinoma (HBC) cell lines series. The 21T series are closely matched pairs of mammary carcinoma cell lines derived from a single patient and are characterized by exhibiting a gradient order of malignancy (21MT> 21NT> 21PT), in addition to a normal mammary epithelial cell line (H16N2), derived from the same patient. The 21MT lines showed the highest expression of AhR protein and mRNA (~20 fold higher than H16N2), and 21NT was medium between 21MT and 21PT. Although TCDD treatment of H16N2 and 21PT have resulted in substantially lower levels of AhR protein, the 21NT and MT cell lines were less responsive to that effect of TCDD. While plasminogen activator inhibitor-2 (PAI-2) levels in 21T series were inherently high in all three lines and less sensitive to TCDD treatment, its levels and the levels of urokinase plasminogen activator (u-PA) receptor expressed in these cells were proportional to their malignancy in direct correlation to the AhR levels. In conclusion, these data identify the AhR as a key regulator in breast cancer progression, and establish a positive correlation between the AhR expression and components of uPA system. While the AhR activation by TCDD is associated with induction of some components of u-PA system, the lack of this response in the advanced tumorigenic 21NT and 21MT lines suggests that the receptor over-expression and its PAH-dependent activation are probably different pathways.

303 CONNEXIN PLAQUE EXPRESSION IN RELATION TO PROLIFERATIVE RESPONSE TO NON-GENOTOXIC CARCINOGENS.

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Gap junction intercellular communication (GJIC) is thought to play an important role in maintaining tissue homeostasis by influencing cell growth and cell death. Disruption of GJIC *via* down-regulation of connexin (Cx) proteins has been sug-

gested as a potential mechanism involved in the action of many non-genotoxic carcinogens. We have investigated the time- and dose-dependent effects of non-genotoxic carcinogens on connexin plaque expression and cell proliferation in target and non-target tissues. Rats were treated with TCDD (0.0025, 0.025, 0.25 μg/kg), Wy-14, 643 (50 mg/kg), chloroform (18, 90, 180 mg/kg) and *p*-dichlorobenzene (DCB) (30, 150, 300 mg/kg) for 3 and 28d. Tissue sections were immunolabelled for Cx26, Cx32 and PCNA. Treatment with Wy-14, 643 led to a significant increase in PCNA-labelled hepatocytes (indicative of proliferation) and reduction in the number and total area of Cx-labelled plaques, quantified in random regions. Similarly, administration of TCDD resulted in decreased Cx expression in both liver and thyroid, but without changes in cell replication. No effects were seen in kidneys (non-target organ) of TCDD and Wy-14, 643 treated animals. In contrast, a dose-dependent reduction in Cx32 and Cx26 levels was detected in cortical epithelial cells of rats treated with the male rat kidney carcinogens chloroform and DCB at both time points, while changes in proliferation were seen only following 3d treatment with 180 mg/kg chloroform and 28d treatment with 300 mg/kg DCB. In the liver (not target organ), chloroform and DCB led to a transient increase in proliferation without effects on Cx expression. In summary, modulation of Cx expression was observed in target organs following short-term treatment with non-genotoxic carcinogens. No changes were seen in non-target organs analysed. While aberrant expression of Cx plaques did not correlate with proliferative response to carcinogen treatment, the interaction of both processes may be important in non-genotoxic carcinogenesis and determination of threshold effect levels.

304 QUANTITATIVE PROTEOMIC ANALYSIS OF MOUSE LIVER RESPONSE TO THE PEROXISOME PROLIFERATOR DIETHYLHEXYLPHTHALATE (DEHP).

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Peroxisome proliferators (PPs) are a diverse group of chemicals that cause hepatic proliferation, suppression of apoptosis, peroxisome proliferation and liver tumours in rodents. The biochemical response to PPs involves changes in the expression of peroxisomal β-oxidation enzymes and fatty acid transport proteins such as acyl CoA oxidase (ACO) and liver fatty acid binding protein (L-FABP). The response to PPs is mediated by the peroxisome proliferator activated receptor α (PPARα) and the livers of PPARα null transgenic mice do not develop tumours in response to PPs. In order to identify the molecular pathways underlying the adverse effects of PPs in rodent liver, we carried out 2D differential gel electrophoresis (2D-DIGE) to provide quantitative proteomic analyses of diethylhexylphthalate (DEHP) treated wild type or PPARα null mouse livers. Since tumourigenesis is both PP and PPARα dependent, analyses were focused on these changes. Fifty nine proteins were identified where altered expression was both PPARα and PP-dependent. In addition, 6 proteins regulated by the deletion of PPARα were identified possibly indicating an adaptive change in response to the loss of this receptor. The proteins we identified as being regulated by PPARα are known to be involved in lipid metabolism pathways, but also in amino acid and carbohydrate metabolism, mitochondrial bio-energetics and in stress responses including several genes not previously reported to be regulated by PPARα. These data provide novel insights into the pathways utilised by PPs and may assist in the identification of early markers rodent nongenotoxic hepatocarcinogenesis.

305 CHARACTERIZING THE MOUSE LIVER TUMOR RESPONSE THROUGH EXPRESSION PROFILE ANALYSIS.

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Human carcinogen risk is commonly assessed in the B6C3F1 mouse strain, a cross between a hepatocarcinogen-resistant (C57BL/6) and a hepatocarcinogen-sensitive (C3H/He) strain. Other mouse strains are known to vary in their hepatocarcinogen sensitivity as well. Although a number of hepatocarcinogen sensitivity/resistance (hcs/hcr) loci have been mapped, the molecular basis for these sensitivity variations is unknown. We hypothesize that expression differences of key genes in these loci are responsible for some of the observed strain variation. Hepatic transcript profiles of 7 mouse strains (A/J, AKR/J, B6C3F1, C3H/HeJ, C57BL/6J, C57BR/cdJ, DBA/2J) and profiles of chemically (DEN, Wy-14, 643) induced hepatic adenomas were obtained using the Atlas Mouse Cancer 1.2 array (Clontech). The Phenotypic Trait Value (PTV) method, which correlates phenotypic data with gene expression levels, was used to analyze these data. Identified in the analysis were 144 genes related to hepatocarcinogen sensitivity, 45 genes related to the rate of appearance of hepatic foci (conversion rate), 138 genes related to the rate of increase in hepatic foci size (propagation rate), 264 genes related to DEN-induced adenomas and 144 genes related to Wy-14, 643-induced adenomas. Of these genes, 78 are located near hcs/hcr loci and may play a role in differential tumor susceptibility. In addition, many of these genes fall into classes of genes related to malignant transformation (e.g., cell cycle regulators). In summary, we used multiple data sources to focus on genes whose expression most likely explains the differences between resistant and susceptible mouse strains at the molecular level. Knowing how these differences determine sensitivity to a chemical carcinogen will lead to a better understanding of the mechanisms of hepatocarcinogenesis in mice and a better understanding of the relevance of the mouse liver tumor response in human risk assessments.

306 ACRYLONITRILE EFFECTS ON GENE EXPRESSION IN RAT BRAIN AND GLIAL CELLS.

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Chronic exposure to acrylonitrile induces astrocytomas in rats. While the mechanisms for acrylonitrile-induced carcinogenicity are not completely understood, previous studies have shown an induction of oxidative stress in acrylonitrile treated rat brain and rat glial cells. In the present study, we investigated gene expression changes in rat brain following treatment with 200 mg/kg acrylonitrile for 14, 28, and 90 days and in glial cells treated with 25, 50 or 75 mg/ml acrylonitrile for 24 hours. Using cDNA array technology, an up-regulation in several genes associated with cell growth regulation (c-myc, c-jun, egr-1, p27kip2, IL-2 and gadd45 protein) were observed in rat brain following treatment with acrylonitrile for 14 days. After 28 and 90 days of treatment with acrylonitrile, expression of these genes was lower than that seen at 14 days and gene expression of c-fos and c-jun was lower than control. An elevation in NF-κB pathway was seen at all time points. In acrylonitrile-treated glial cells, an increase in expression of genes involved in NF-κB signaling were up-regulated while gene regulation of cell proliferation pathways were down-modulated and genes involved in the apoptotic pathway were up-regulated at 24 hours. Since NF-κB activation is regulated post-transcriptionally, by translocation of the transcription factor from the cytoplasm to nuclei, a minor change in mRNA proteins may represent a significant usage (or activation) of NF-κB early after acrylonitrile treatment. Activation of NF-κB by acrylonitrile was therefore confirmed in glial cells with a reporter gene assay utilizing glial cells transfected with an NF-κB plasmid. The results provide important information on the genes modulated in the brain by treatment with acrylonitrile. Since many of the observed modulated genes are regulated by reactive oxygen species, it is likely that these changes in gene expression are due to acrylonitrile-induced oxidative stress.

307 ALTERED GENE EXPRESSION ASSOCIATED WITH FURAN INDUCED CHOLANGIOFIBROSIS IN THE RAT.

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Cholangiocarcinoma (CC) is the most common primary hepatic malignancy in humans after hepatocellular carcinoma, comprising 15% of liver neoplasms. Human intrahepatic CC is a usually fatal malignant neoplasm thought to originate from biliary epithelium and associated cholangiofibrosis. We have studied gene expression changes to help characterise stages in the development of CC in the rat. Rats were dosed with furan at 35mg/kg daily for 3 months and also with 1 month 'off-dose' prior to necropsy to characterise persistent changes. Lesions with an elevated proliferating cell nuclear antigen (PCNA)-labelling index, and associated cell populations with increased wild type p53, phosphorylated c-jun and cdk-1 protein expression, were compared to livers of control rats using the Affymetrix Rat Genome U34A Array. 310 genes were over-expressed in both 3 and 3+1 month livers compared to control. 105 genes showed a reduced expression compared to control. A number of these changes clearly reflect the shift in major cell type and fibrosis associated with the lesion. However a sub-population of genes with established roles in proliferation was altered in expression. These are currently being studied further using real time quantitative PCR (TaqMan) in conjunction with Laser Capture Microdissection (LCM) to establish those gene expression changes that distinguish the hyperplastic and metaplastic regions from normal biliary epithelium.

308 MALIGNANT TRANSFORMATION BY MNNG AND ANTITRANSFORMATION BY ARSENIC OR METAL MIXTURE IN KERATINOCYTES.

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In order to evaluate the carcinogenic potentials for chemicals such as arsenic (As) and other metals, we have identified molecular markers potentially involved in the process of carcinogenesis in keratinocytes. We have demonstrated that treatment

with MNNG enhanced transformation of the immortal human keratinocyte cell line, RHEK-1. In contrast, As alone or in mixtures of As, Cd, Cr, and Pb inhibited this process. Microarray analysis showed unique gene expression patterns in RHEK-1 cells treated with the chemicals/mixtures. To explore markers that may be involved in the enhancement or inhibition of malignant conversion, 9 genes commonly affected by the MNNG and As/mixture treatments were examined for expression in more detail by kinetic PCR. These 9 genes were mitogen-responsive phosphoprotein DOC2, vimentin, superoxide dismutase 1(SOD1), macrophage inhibitory cytokine 1(MIC1), glutathione (GSH) synthase, bikunin, multiple tumor suppressor 1(MTS1), UV excision repair protein RAD23A, and bone morphogenetic protein 4(BMP4). Four genes were altered in their expression in a manner consistent with a role in the transformation process. MIC1, which was induced 8-fold by MNNG and suppressed up to 12-fold by both As and the mixture, encodes a TGFβ-like protein and may be involved in malignant process. Two tumor suppressor genes, bikunin and MTS1, and TGFβ-like BMP4 were suppressed by MNNG; these same genes were induced by both As and the metal mixture. GSH synthase, SOD1 and RAD23A were all induced ≥ 2-fold by chronic treatment of RHEK-1 with As and the mixture; these changes are likely a stress response to oxidative or DNA damage. We are currently analyzing gene expression changes in RHEK-1 transformed by other agents such as dioxin or X-ray. Identification of a common battery of genes altered during progressive transformation of RHEK-1 should aid in a mechanistic understanding of this process.

309 GENOMIC INSTABILITY AND GENE COPY NUMBER CHANGES IN THREE CELL LINES COMMONLY UTILIZED IN TOXICOLOGICAL STUDIES.

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Most cancer lines are genetically unstable and undergo changes in gene expression and genomic copy number. This study examines genetic alterations in these cells, that are linked to tumor progression. We performed comparative genomic hybridization (CGH) on three cell lines commonly employed in cancer biology and toxicological studies. CGH is a molecular cytogenetic assay that screens for DNA copy number changes globally within a complete genomic complement and maps these changes onto normal chromosomes. This technique is based on the simultaneous hybridization of differentially labeled (fluorescent labeled) total genomic tumor DNA and normal reference DNA onto human metaphase chromosomes. Image analysis generates a "copy number karyotype" for the cell line, indicating relative gains and losses of segments along the length of each chromosome. MCF-7 and 47-D represent a well-established system for studying the biology of breast cancer cells, whereas SY5Y cells typifies a neuronal system. The copy number karyotype found in T47-D and SY5Y cells were identical in passages 7 and 34 and 27 and 87 respectively. Whereas in MCF-7 cells there were clear differences in genomic copy number changes between passage number 30 and 85. The genomic gains > 3copies in p85 as when compared to p30 include chromosome region 7q22, 8q21-23, 14q23-24, 17q22-23, 20q13, 22q and the genomic losses include -5p, -8p, -13q. Several of these loci harbor oncogenes and tumor suppressor genes, for example, HTK-hepatoma transmembrane kinase, BRCA1-familial breast cancer predisposition gene, BCAS1-breast cancer amplified sequence1, cadherin and Rb. Genetic alterations in these or other genes in these loci may contribute to tumor progression. Further characterization of these loci by complementary molecular approaches will help elucidate the genes involved in tumor progression in this cell line.

310 TEMPORAL CHANGES IN THE LEVELS OF XENOBIOTIC-METABOLIZING ENZYMES AND ALIPHATIC HYDROCARBONS IN RATS EXPOSED TO A PETROLEUM CRUDE OIL.

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In Alberta, Canada, some agricultural land sites are also used for petroleum production. This study was done to assess the temporal effects of Pembina Cardium crude oil (PCCO) in rats. Pair-wise groups of rats (6/group) were exposed to either PCCO or potable water (control) by gavage at 2.5 ml/kg on days 0, 7, and 14. The paired groups were then sacrificed on post-exposure days 1, 7, 10, 14, 21 and 28, and selected tissues were analyzed for biochemical parameters, and aliphatic hydrocarbons (n-alkanes). Temporal assessment of cytochrome P450 (CYP)-linked monooxygenases in both groups showed that on day 1 of post-exposure, the PCCO-exposed rats had a marked induction of ethoxyresorufin-O-deethylase (EROD), a CYP 1A1-linked enzyme, (15-20 fold), and pentoxyresorufin-O-dealkylase (PROD), a CYP 2B-linked enzyme, (5-8 fold) in liver. In lungs, the EROD activity was also greatly elevated (5-7 fold), but the activity of PROD was markedly reduced (50-60%). In the subsequent period, these enzymes activity in PCCO-exposed rats gradually returned to levels observed in the control rats. Exposure to PCCO also caused a marked increase in the total concentration of n-alkanes (C_{10} - C_{19})in body fat (3-5 fold). The post-exposure decrease in the levels of n-alkanes from the body fat was gradual in nature; however, their levels in liver decreased markedly in the early post-exposure phase. The differential systemic responses of CYP-linked enzymes and n-alkanes in PCCO-exposed rats indicated a close link between the levels of hydrocarbon compounds and the related monooxygenases.

311 TRANSLOCATION OF BIOMARKER CHEMICALS INTO SHEEP TISSUES AFTER ORAL EXPOSURE TO CRUDE OIL.

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Food safety is a high priority in Alberta, Canada. The landscape in Alberta is often shared by the petroleum and livestock industries providing opportunity for ruminants to be exposed to oilfield substances. The implications of petroleum ingestion on food safety do not appear to be known. Sheep (n=11) were administered 10 mL of Pembina cardium crude oil (PCCO)/ kg body weight or 10 mL of potable water on study days 0, 14, 28 and 42. Blood for clinicopathology was collected across study days. The sheep were necropsied on study day 43 and fat, brain, liver, kidney and skeletal muscle were collected for hydrocarbon analyses. The n-alkanes (C_{10} to C_{19}) were extracted from tissues and identification and quantification were made by gas chromatography - mass spectrometry techniques. The total concentration of the C_{10} to C_{19} n-alkanes found in tissues were omental fat 61.3±5.37 (SE) ppm, perirenal fat 70.2±7.08 ppm, tailhead fat 60.1±6.67 ppm, bone marrow fat 41.4±5.90 ppm, liver 4.7±0.43 ppm, brain 0.6±0.08 ppm, and kidney 0.1±0.08 ppm. The C_{10} to C_{19} n-alkanes were below detection level (<0.1 ppm) in skeletal muscle. Clinicopathologic observations include a leucocytosis primarily due to a neutrophilia. Lesions were observed in the lungs during necropsy. This study showed that oral exposure of sheep to crude oil has potential of being a food safety issue.

312 TOXICOPATHOLOGY AND IMMUNOTOXICOLOGY OF MULTIPLE EXPOSURES TO DIESEL AND CRUDE OILS IN CATTLE.

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The study being reported was part of a large study on the subchronic toxicology of crude and refined petroleum in cattle. Forty cattle (n=8/group) were gavaged on study days 0, 7, 14, 28 and 42 with 10 mL potable water (PW)/kg body weight (BW), 5 mL winter diesel oil No. 2 (WDO-2)/kg BW, or Pembina cardium crude oil (PCCO) at 2.5, 5 and 10 mL/kg. Repeated clinicopathology and immunologic determinations were done from study days -14 to study day 48. The cattle were necropsied at 22±7 days after the last dose. Statistical analyses showed differences between control (PW treated animals) and animals treated with PCCO or WDO-2. Significant hematologic changes were a decrease in Hct, Hb and differential counts for lymphocytes, basophils and eosinophils as well as significant increases in platelet numbers, the neutrophil/lymphocyte ratio and in differential counts for neutrophils. Significant changes were also observed in serum iron, LDH, AP, GGT, albumen, creatinine, glucose, bilirubin, cholesterol, triglycerides, Ca and Mg. Oil treatments stimulated neutrophil random migration, and specific neutrophil chemotaxis was increased after the first oil treatment but reduced after dose 4. Compared to pre-dosing levels, oil treatment (at dose 3) resulted in a higher proportion of CD3+, CD2+, CD4+ and CD25+ cells and lower proportion of δγ T cells, CD14+ and B cells in peripheral blood, suggesting immune activation and recruitment of some cell types to peripheral sites. Macro- and microscopic tissue lesions linked to treatment with WDO-2 and PCCO were not observed. Repeated oral exposure to petroleum products induced significant changes in both clinicopathologic and immunopathologic parameters without producing significant macro- or microscopic tissue lesions.

313 MAMMALIAN TOXICITY OF NAPHTHENIC ACIDS DERIVED FROM THE ATHABASCA OIL SANDS (AOS).

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The mammalian toxicity of naphthenic acids isolated from AOS tailings was investigated by orally administering these compounds to female Wistar rats. Naphthenic acids are natural constituents of petroleum, and can represent an important waste

contaminant generated during petroleum processing. Naphthenic acids are known to be highly toxic to aquatic organisms, but effects on terrestrial animals are largely unknown. Subchronic toxicity testing revealed a number of significant effects were associated with a dosage of 60 mg/kg/d naphthenic acids for 90 d. Dosed animals had significantly lower (-9%) body weights than controls throughout the test, despite comparable food consumption. Some terminal organ weights were higher in this dose group, particularly that of liver, which was 36% higher than in controls. Plasma biochemistry was significantly affected, with total cholesterol of dosed (60 mg/kg/d) animals 43% lower than controls. In contrast, this dosage caused amylase to rise 33% above control values. Reproductive toxicity testing demonstrated dramatic effects on female fertility at an oral dosage of 60 mg/kg/d naphthenic acids during pre-breeding, breeding, and gestation. While control and low dose (6 mg/kg/d) animals achieved 93 and 100% reproductive success, respectively, only 7% of females dosed at 60 mg/kg/d successfully bore a litter. Total cholesterol of the latter group was 30% lower than controls. Mating and ovulation were comparable amongst control and dose groups, while fetal malformations were not apparent in any offspring. Results suggest that the dose-related infertility may be associated with poor embryonic implantation, an effect that might be secondary to depressed sex hormone production requiring cholesterol as a precursor. Current research is evaluating this mechanism, and whether naphthenic acids, structurally similar to cholesterol metabolites, directly alter the rate of cholesterol biosynthesis *in vivo* by means of a possible allosteric effect.

314 A COMPARISON OF THE EFFECTS OF SHORT TERM ORAL EXPOSURE TO 2, 4, 6-TRINITROTOLUENE (TNT), 1, 3, 5-TRINITRO-1, 3, 5-TRIAZINE (RDX) AND CYCLOTETRAMETHYLENE-TETRANITAMINE (HMX) IN NORTHERN BOBWHITE QUAIL *(COLINUS VIRGINIANUS).*

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Potential risk to wildlife from exposure to the explosives, 2, 4, 6-trinitrotoluene (TNT), 1, 3, 5-trinitro-1, 3, 5-triazine (RDX) and cyclotetramethylene-tetranitamine (HMX) has been a concern throughout numerous military installations where it has been found in the soil. To date, limited data have been generated on the effects of oral exposure to TNT and no data have been generated for RDX and HMX in an avian species. Acute oral exposure and sub-acute dietary exposure to TNT, RDX and HMX was therefore evaluated in a species commonly found at military installations, the Northern Bobwhite *(Colinus virginianus).* Adult male and female quail (N = 1/ sex/dose) were given acute oral doses of each of the compounds at 4782, 3188, 2125, 1417, 945, 630, 280 187, 125 mg/kg and evaluated for 14 days duration. Acute lethal toxicity was observed at 2125 mg/kg with TNT for both genders. RDX doses of 280 mg/kg and 187 mg/kg were acute lethal doses in the male and female, respectively. Interestingly, HMX failed to induce an acute lethal dose during the initial ALD study. Even in a repeated study with concentrations as high as 10, 760 mg/kg, acute lethal toxicity to HMX could not be determined. Blood analysis with HPLC showed a strong correlation between acute toxicity and quantity of compound found in the blood. This suggests that these compounds varied in their ability to be absorbed into bird's blood stream. Additionally, fecal analysis of the compounds showed an inverse correlation to the severity of toxicity. Further, fasting status of the birds also seemed to impact the absorption. These data suggest that Northern Bobwhite appear to be less sensitive than mammals to acute oral exposures of TNT, RDX and HMX.

315 CYTOCHROME P450 MODULATION AND ANTIOXIDANT MITIGATION OF 2, 4, 6-TRINITROTOLUENE TOXICITY.

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Toxicity of 2, 4, 6-trinitrotoluene (TNT) and three of it's degradation products, 2, 4-diamino-6-nitrotoluene (DANT), 2-amino-4, 6-dinitrotoluene (2-ADNT), and 1, 3, 5-trinitrobenzene (TNB) was determined utilizing two aquatic invertebrates. Differential toxicity of the degradation products was observed in two organisms, Neanthes areanceodentata, a marine polychaete, and Hyalella azteca, a freshwater amphipod. Implications of the differential toxicity in these aquatic organisms derive specific challenges when conducting an ecological risk assessment. To gain a better understanding of the mechanisms responsible for the changes in toxicity, investigations were conducted through a series of toxicity experiments focusing on oxidative stress and metabolism by cytochrome p450. These mechanisms were studied by exposing the organisms to TNT in combination with a cytochrome p450 inducer and inhibitor and two antioxidants. Toxicity of TNT was increased in a time and concentration dependent manner following exposure to clotrimazole, suggesting inhibition of cytochrome p450 decreases the metabolism and elimination of TNT. BNF modified the toxicity of TNT in a U-shaped dose and time dependent manner. BNF at 0.125 mg/l significantly decreased the toxicity of TNT suggesting that increased cytochrome p450 activity increases the elimination of TNT. Ascorbic acid and tocopherol mitigated the toxicity of TNT to H. azteca. Pre-exposure to ascorbic acid (0.13, 0.44, 0.88, and 1.32 mg/L) significantly decreased mortality associated with a 96-hr exposure to 3.0 mg/L TNT. Pre-exposure to tocopherol decreased mortality in a U-shaped dose-dependent trend; mortality was the lowest at 0.125-mg/L tocopherol and 3 mg/L TNT. Based on these data it is suspected that TNT elicits its toxicity through oxidative stress. These results demonstrate the sensitivity of aquatic invertebrates may be the result of differences in metabolic capacity (cytochrome p450 activity) and antioxidant capacity of the organism.

316 QUANTIFICATION OF ATRAZINE (ATRA) AND DIAMINOCHLOROTRIAZINE (DACT) IN RAT WHOLE BLOOD UTILIZING GC/MS/SIM.

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Atrazine (ATRA) is a selective chloro-s-triazine herbicide for broadleaf and grassy weed control in corn, sorghum, sugarcane, and other crops. Diaminochlorotriazine (DACT) is the major urinary metabolite seen (64-67% of total) in *in vivo* studies utilizing radiolabeled ATRA administered orally. We are developing a suite of analytical methods to assist pharmacokinetic model development in rodents and humans, to assess human exposures *via* examination of urine metabolites, and to assess protein binding of these compounds in blood and hair [refer to Ruble et al, UTILIZATION OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL TO PREDICT DOSE-DEPENDENT GSH DEPLETION AFTER ATRAZINE TREATMENT, 41ST Annual SOT National Meeting poster session]. In this work, we have developed methods to measure ATRA and DACT in blood. Rat whole blood was spiked with ATRA and DACT and cleaned up with solid phase extraction (SPE). The SPE eluate and standards were derivatized with methyl iodide and tetra-butyl ammonium hydroxide (TBAOH), yielding the dimethyl derivative of ATRA and the tetra-methyl derivative of DACT. Derivatization is not needed for ATRA but is necessary for accurate quantification of DACT by removing undesirable column interactions and thus improving peak symmetry. Samples and standards were analyzed with Gas Chromatography/Mass Spectrometry (GC/MS) in Specific Ion Monitoring (SIM) mode. Results showed excellent recoveries of ATRA and DACT with detection limits near 100 ppb. These methods have also been employed to determine time course concentrations of chloro-s-triazines in blood following dosing in rats. This work was supported by STAR grant R-828610-01-0 and HICAHS grant U07/CCU807121-10.

317 DOES BINDING TO ALBUMIN MODULATE THE BIOLOGICAL EFFECTS OF PERFLUOROOCTANE SULFONIC ACID?

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Perfluorooctane sulfonic acid (PFOS) has been demonstrated to accumulate in the liver and blood of exposed organisms. The potential for these detergent-like molecules to interfere with normal ligand/protein interactions in blood is of concern given the importance of many of these interactions, particularly those involving the transportation and action of hormones. Due to concern over any compounds that might modulate the endocrine system, PFOS binding to serum proteins was investigated. The ability of PFOS to displace a variety of steroid hormones from their specific binding protein sites in serum was measured. PFOS had only a weak capacity to displace the steroid hormones estrogen and testosterone from carp serum steroid binding proteins. The effects on corticosterone binding globulin in bird species (chicken and bald eagle) occurred at the least PFOS concentrations. Corticosterone displacement potency increased with increasing chain length and sulfonic acid compounds were more potent than those with a carboxylic acid functional group. The estimated PFOS concentrations to cause these effects were 320]1M or greater, equivalent to a serum concentration of greater than 160 ppm. Using mass spectrometry and *in vitro* binding assays, PFOS was demonstrated to bind strongly to bovine serum albumin (BSA). The combined results from these studies demonstrate that BSA binds only one or two PFOS molecules per protein molecule. This binding ratio is relatively low compared to the known 8 - 11 binding sites for other ligands such as fatty acids. Concentrations of PFOS required to saturate this binding would be in excess of 50 to 100 mg/L. Therefore, based on current environmental concentrations it is extremely unlikely that PFOS would cause significant displacement of hormones from their serum binding proteins in wildlife.

318 ONE FISH, TWO FISH, RED FISH, BLUE FISH: THE FISH QUALITY INDEX AS A RISK COMMUNICATION TOOL.

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Many people, but particularly subsistence fishermen, are at high risk for methyl mercury toxicity because of their consumption of contaminated fish. Often health risks of Persistent Bioaccumulative Toxicants (PBT) such as methyl mercury, PCBs or Dioxins are underestimated because of their amplification in the food chain resulting in toxicity even though ambient levels of these PBT are within acceptable limits. Two concerns have developed from this situation (1) most of the affected groups have not been identified, and (2) means of effectively communicating the possible risk to the affected groups do not readily exist. We are defining regions and specific lakes and rivers that have high levels of consumption of fish, and where high PBT concentrations in water can occur. We are also identifying populations of subsistence fishermen or other high-risk groups who consume large quantities of fish from these waters. The final product will be a user-friendly risk communication tool (the Fish Quality Index), which is a color-coded pictogram for various fish species (for example; green ones are safe to eat, even at subsistence consumption levels, yellow fish are safe once a month while red ones are safe only if eaten less than once a year). This map-based tool transcends language and literacy boundaries and should inform anglers of the relative health risks according to the species of fish that they regularly consume in an easy to understand format. It has the benefit of steering people toward safer lakes and streams and safer species of fish. In most lakes we've examined, there are safe species of fish and unsafe species of fish in the same lake, depending mostly on the trophic level of the fishes food supply. A pilot of the fish quality index has been developed for several New England States, and is currently being tested by several groups. It will eventually be available on the internet, and on CD-ROM, but the individual maps shown at the presentation can be printed in wallet-size or larger formats.

319 EXPOSURE AND HEALTH EFFECTS OF INHALED FLORIDA RED TIDE BREVETOXINS ON HUMANS DURING A RED TIDE EVENT.

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The toxigenic marine dinoflagellate *Karenia brevis* is responsible for red tides in the Gulf of Mexico. The neurotoxic agents produced in red tides, the brevetoxins, cause asthma-like symptoms in beach-going populations. During the Fall of 2001, an extensive Florida Red Tide was studied off the coast of Sarasota Florida. Environmental data including K. brevis counts in the water and the amount of toxin present, toxin in aerosols transported on-shore, subsequent exposure of humans through respiration, and both throat swab and epidemiologic data on occupationally-exposed individuals were collected. These data were supported by GPS and meteorological measurements. Brevetoxin enzyme-linked immunoassays (ELISA) quantified total toxin on-site, and ELISA and LC-coupled mass spectrometry were used in the Lab. . During the 5-day study, toxin concentrations were measured at six different locations and ranged from 20 ng/mL to 400 ng/mL. K. brevis cell counts ranged from 1, 000 to 15 million cells/L. Impact air sampler filters collected 80-467 ng/sq cm toxin in airborne particles. Human symptoms of sneezing, eye irritation, and coughing were experienced by the lifeguard subjects and scientists. Toxin levels and symptoms were inversely correlated with distance from the shoreline. The severity of symptoms correlated with the measured concentrations of toxin from personal and high volume air samplers. Throat and nasal swabs of both subjects and environmental scientists revealed increased numbers of inflammatory cells. Phagocytic cells stained by brevetoxin ELISA were inconclusive.

320 PARALYTIC SHELLFISH TOXINS: INTERACTIONS WITH MAMMALIAN DETOXIFICATION ENZYMES.

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Paralytic shellfish toxins (PST) are important marine phycotoxins associated with paralytic shellfish poisoning which is a significant food safety problem due to consumption of seafood contaminated by this suite of toxins. In Hong Kong, the C1 and C2 toxins (C1/2) and gonyautoxins 2 and 3 (GTX2/3) are prevalent members of PST found in its coastal waters. Although much detail is known about the mechanism of action of PST as sodium channel blockers, little is known about their metabolism/detoxification in mammalian systems. Studies were undertaken to examine the interaction of PST with mammalian detoxification enzymes. Incubation of these toxins with enzyme preparations containing Phase I and Phase II enzymes from rat and mouse livers resulted in no observable changes to these toxins. However, administration of these toxins to mice followed by assaying for various detoxification enzymes resulted in the reduced activity of ethoxyresorufin-O-deethylase (EROD), penthoxyresurofin-O-depenthylase (PROD), superoxide dismutase (SOD) and glutathione peroxidase (GPx). No change in activity was found for glutathione-S-transferase (GST), catalase (CAT) and glutathione disulfide reductase (GR). These results indicate that although the investigated PST are not metabolized, they can suppress some critical detoxification enzymes and may have adverse effects on mammalian physiology. (Supported by HKUST6102, RGC)

321 IMMUNE FUNCTION OF CRYO-PRESERVED AVIAN PERIPHERAL WHITE BLOOD CELLS: A POTENTIAL BIOMARKER OF CONTAMINANT EFFECTS IN WILD BIRDS.

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There is a need to develop sensitive biological markers of contaminant-induced effects in wildlife. Immune function may prove a useful marker for toxic effects because its complexity renders it sensitive to contaminant exposure. However, there are limitations of collection and transport of immune cells in the field. Here we report the development of methods to assess immune function in wild birds, using cryo-preserved chicken peripheral white blood cells (WBCs). We determined that cryo-preservation caused a slight but measurable decrease in cell viability (99% +/-.2SE for fresh cells and 84%+/-2SE for cryo-preserved cells (n=8)). There was no reduction of viability between cells that were cryo-preserved for less than 10 days (88%+/-4SE) and greater than 50 days (89%+/-1SE) (n=6). Importantly, cryo-preserved cells responded well to tests of immune cell function (mitogen-induced cell proliferation and macrophage phagocytosis). To validate the use of cryo-preserved white blood cells to assess contaminant effects, we used these tests on cells exposed *in vitro* to the poly aromatic hydrocarbon 9, 10, Dimethyl-1, 2-Benz-anthracene (DMBA), a well-known immuno-suppressant. We found that DMBA significantly ($p<0.05$) reduced the ability of cells to proliferate and ingest yeast. We tested the applicability of these methods on wild American Coots *(Fulicia americana)* by cryo-preserving WBC for a period of up to 10 months (average viability of 75% +/-11SD, n=10) and performed immune function tests (proliferation response ranged from 224 to 583% above the non-stimulated cells and the phagocytic ability of macrophages ranged between 15-88%). This research is valuable because it provides a technique to collect samples non-lethally with a single blood sample, as well helping to establish the use of immune function for the assessment of contaminant exposure.

322 DEVELOPMENT OF ANTIBODIES TO SATRATOXINS AND APPLICATION TO ENZYME-LINKED IMMUNOSORBENT ASSAY.

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Although macrocyclic trichothecene mycotoxins produced by *Stachybotrys chartarum* have been associated with numerous outbreaks of indoor illness, no simple method exists for the detection of these toxins in environmental samples. The goal of this study was to produce specific antibodies to the macrocyclic trichothecene mycotoxin, satratoxin G (SG), and employ these in an enzyme-linked immunosorbent assay (ELISA). SG was derivatized to its hemisuccinate and coupled to bovine serum albumin (BSA-SG) for use as an immunogen. Three rabbits were immunized by intradermal injections of the immunogen followed by a single booster injection at week 18. SG-specific antibodies were detected as early as 4 wks of immunization and high titers still detectable 38 wks later. A direct competitive ELISA employing microtiter well-adsorbed SG antibodies and SG-hemisuccinate coupled to horseradish peroxidase (HRP-SG) was developed and it could detect as little as 0.1 ng/ml of SG. The concentrations of SG, satratoxin H, isosatratoxin F, roridin A, and verrucarin A causing 50% inhibition of HRP-SG conjugate binding were 1.3, 5.4, 3.2, 25.1, and 52.5 ng/ml, respectively, indicating that the antibodies were most specific for SG. Assay sensitivity was largely unaffected by methanol content up to 20% in toxin diluents suggesting that the assay could be applied to methanol extracts of environmental samples. The ELISA was applicable detection of satratoxins

in *S. chartarum* spore extracts. SG antibodies should be useful for identifyinging the toxigenic S. chartarum isolates as well as the rapid detection of SG and other satratoxins in environmental samples from buildings contaminated with this fungus.

323 COMPARISON OF GC/MS AND GC/μECD METHODS ON THE ANALYSIS OF HEXACHLOROBENZENE (HCB) IN RAT BLOOD AND LIVER.

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Hexachlorobenzene (HCB), a persistent environmental pollutant, was once used widely as a fungicide but has been banned since the 1970s. However, because of its accumulation in the food chain and in lipid deposits of animals and man, HCB has been selected for toxicological evaluation by the NIEHS. In the analysis of HCB from rat blood and liver samples from a toxicokinetic study, methods were validated using GC/MS and GC/μECD. For blood, sample preparation of a 100 μL aliquot included homogenization by Polytron, if clotted material was present, followed by hexane extraction. For liver, samples of 0.1, 0.3 and 0.5 g were homogenized by Polytron followed by hexane extraction. For both matrices, HCB concentrations were determined using the peak area ratio, PAR, (Isodrin, 2 μg/mL, internal standard) compared to a spiked matrix standard curve. The GC/MS methods compared the J & W DB-5 to the DB-5MS capillary column (30 m x 0.32 mm, 1 μm film), with the DB-5MS showing less baseline noise. Comparison of GC/MS and GC/μECD separation and detection parameters allowed for the used of GC/μECD for routine analysis of samples in a cost-effective manner. The GC/μECD method used a Restek RTX-5 analytical column (60 m x 0.25 mm, 0.25 μm film). The blood HCB methods were validated using GC/MS at ~1 to 400 ng/mL and for GC/μECD at ~20 to 5000 ng/mL. For liver HCB, methods were validated using GC/MS at ~ 1 to 10, 000 ng/g and for GC/μECD at 4 to 10, 000 ng/g. Actual blood samples from the toxicokinetic study ranged in HCB concentration from 20 to 160, 000 ng/mL, while actual liver samples ranged from 8 to 460, 000 ng/g.

324 4-NONYLPHENOL INHIBITS INOS GENE EXPRESSION IN LPS-STIMULATED RAW 264.7 CELLS BY BLOCKING NF-KB ACTIVATION.

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4-Nonylphenol (NP) is a degradation product of a widely used non-ionic surfactant group, alkylphenol polyethoxylates that are mainly found as an intermediate in the chemical manufacturing industry. In this study, we investigated the effect of NP on the regulation of inducible Nitric Oxide Synthase(iNOS) in murine macrophages. NP alone did not affect the expression of iNOS; in contrast, suppressed the LPS-induced gene expression of iNOS, in a dose-dependent manner as determined by RT-PCR analysis. NO production was assessed by measurement of nitrites in the medium. The level of NO was found to correlate well with a decrease in transcripts of iNOS. Since the promoter in iNOS gene contains binding motifs for NF-kB, the effect of NP on the inactivation of this transcripts factor was determined by transient transfection assay. Employing a transfection and reproter gene expression system with p(NF-kB)3-Luciferase, the treatment of NP produced a dose-dependent inhibition of luciferase activity in RAW 264.7 murine macrophages cell line. These results suggest that suppression of iNOS gene expression by NP might be mediated by the inhibition of NF-kB activation [This work was supported by KFDA Grant (ED 2000-33) and RCPM].

325 EFFECTS OF THE HORMONAL GROWTH SUPPLEMENT TRENBOLONE ON REPRODUCTIVE ENDOCRINOLOGY OF THE FATHEAD MINNOW.

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As part of an ongoing effort to develop and validate a fathead minnow (*Pimephales promelas*) assay to detect chemicals that affect processes controlled by estrogens and androgens, we determined the effects of trenbolone on reproductive endocrinology of the fish. Trenbolone is a synthetic androgen receptor agonist commonly used as a growth supplement in beef cattle. Reproductively-active animals were exposed for 21 days *via* the water to nominal trenbolone concentrations of 0, 5, 50, 500, 5000 and 50, 000 ng/L. During this time, reproductive performance (fecundity, fertility, hatch) of the fish was monitored daily. At conclusion of the assay, a number of parameters related to endocrine function (plasma steroid and vitellogenin concentrations, gonadal condition) were assessed in the animals. Reductions in fecundity of the fish, and masculinization of the females were observed at concentrations of 50 ng trenbolone/L and greater. Masculinization was expressed as induction of nuptial tubercles, structures normally observed only on the heads of reproductively-active males. While these data demonstrate that trenbolone is a potent androgen in fish under laboratory conditions, an assessment under relevant field conditions would be required to evaluate whether or not the observed responses might occur in wild populations of fish. *This abstract does not necessarily reflect EPA policy.*

326 EFFECTS OF THREE STEROIDAL ESTROGENS ON REPRODUCTIVE PARAMETERS IN A FISH, *TAUTOGOLABRUS ADSPERSUS.*

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Estradiol (E_2), ethynylestradiol (EE_2) and estrone (E_4) are steroidal estrogens that are released into the aquatic environment in sewage treatment effluent. To determine whether these estrogens impact reproductive parameters in a model fish species, actively-reproducing cunner (*Tautogolabrus adspersus*) were exposed in the laboratory by implanting estrogen subcutaneously in a slow-release matrix. Four tanks with 3 females and 2 males each were used per treatment. Treatments were control (matrix only) and 0.05, 0.5 or 2.5 mg/kg of each estrogen. Egg production (per gram female), viability and fertility were determined daily. Pre-exposure egg production was not significantly different among experiments, averaging 283 eggs/day. Pre-exposure egg viability ranged from 17-20%, while egg fertility ranged from 25-52%. In fish exposed to E_2, egg production in the high treatment was 108±22 compared with 174±46 in control, but this difference was not significant. In fish exposed to EE_2, egg production in the high treatment (28±22) was significantly lower than in control (107±28). E_4 treatment gave no significant differences in egg production, although egg production in the high (99±82) and medium (104±56)treatments was lower than low (188±46) or control (184±104) treatments. Neither mean %viability nor %fertility was significantly different than control in any estrogen treatment. Although GSI was lower in females treated with the 2.5 mg/kg concentration of all estrogens than in other treatments, the decrease was not significant. Average concentrations of vitellogenin (vtg) in control males from the three experiments ranged from 0.0 to 0.5 mg/ml plasma. In males from the highest concentration of EE2, average concentration of plasma vtg (480 mg/ml) was approximately forty times that in the high E_2 treatment (12 mg/ml) and nine times that in the high E_4 treatment (53 mg/ml). Overall, only EE_2 significantly affected mean daily egg production of female cunner. All estrogens elevated plasma vtg in males, but EE_2 notably more so than the others.

327 ALTERATION OF P-GLYCOPROTEIN (PGP) EXPRESSION IN CATFISH BY SELECT ENVIRONMENTAL TOXICANTS.

K. M. Kleinow, P. Uppa, E. Holmes and Y. Zhang. *Comparative Biomedical Sciences, School Veterinary Medicine, Louisiana State University, Baton Rouge, LA.* Sponsor: M. James.

Pgp has been shown to modulate xenobiotic bioavailability and facilitate excretion by efflux transport. Although Pgp in aquatic species is known to be up and down regulated in the environment the etiology of these changes have not been clearly defined. These studies examined Pgp protein expression in catfish following exposure to chemical agents eliciting endocrine disruption, and oxidative stress as well as representative polyaromatic hydrocarbons and flavonoids. Separate groups of catfish were dosed IP daily for 6 days with 2mg/kg of nonylphenol ethoxylate (NPE), quercetin (Q), hydrogen peroxide (HP), methoxychlor (MC) or control vehicle(C). Plasma membranes of liver and intestinal sections were prepared by selective differential centrifugation methods and subjected to electrophoresis, western blotting and immunostaining with the Pgp C219 monoclonal. Pgp was visualized and then quantitated by densitometry. Hepatic Pgp concentrations were significantly higher than controls for NPE and were significantly lower for the HP treated fish. These changes represented 46 % higher and 59 % lower values than controls, respectively. Treatment with Q or MC did not result in significant changes in Pgp for the liver. Enriched brush border membranes of the proximal and distal intestines demonstrated significantly lower Pgp levels (13.2-211 fold) than livers for all treatments including controls. No significant differences in proximal and distal intestine Pgp concentrations were noted for all treatments. These results demonstrate a differential expression of Pgp between the liver and intestine as well as suggest that select xenobiotics or conditions they elicit may alter expression of catfish Pgp, particularly in the liver. (supported by P42 ES07375).

328 EFFECTS OF THE SELECTIVE SEROTONIN REUPTAKE INHIBITOR, PROZAC, ON THE REPRODUCTION OF JAPANESE MEDAKA.

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The occurrence of pharmaceutical products in municipal wastewater has recently received considerable attention; for the most part, potential aquatic impacts are unknown. Prozac (fluoxetine) is a pharmaceutical agent primarily used to treat neurological disorders. Because of the potential for neuro-endocrine interactions of Prozac in aquatic organisms, Japanese medaka were exposed to aqueous nominal concentrations of Prozac (0, 0.5, 1 and 100 ug/L) with subsequent assessment of reproduction. After 14 days pre-reproductive exposure and 14 days reproductive exposure, an increase in the mean total number of eggs produced was observed at the 0.5 ug/L concentration. Also, there was a decrease in the mean total number of eggs produced at the 100 ug/L concentration. Similar trends were observed in the mean number of fertilized eggs at each concentration, as well as the percentage of fertilized eggs that hatched at each concentration. In addition, there was a trend toward an increased incidence of developmental abnormalities. These reproductive trends were not statistically significant, however, observed trends warrant further studies. Recently Prozac has been identified in wastewater effluent from the United States at concentrations in the ng/L range. Future studies will determine the effect of environmentally realistic Prozac concentrations on reproductive parameters in medaka, as well as commonly used reproductive biomarkers (vitellogenin, plasma steroids, etc).

329 A POTENTIAL WHITE STURGEON HEPATIC CYTOCHROME P450 3A WAS CORRELATED WITH LIVER P, P'-DDE AND PLASMA TESTOSTERONE IN WILD WHITE STURGEON.

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White sturgeon *(Acipenser transmontanus)* are experiencing poor reproductive success in some portions of the Columbia River Basin (USA)which may be related to organochlorine contaminants. Previous studies by our group showed that liver 1, 1-dichloro-2, 2-bis(p-chlorophenyl) ethylene (p, p'-DDE) concentrations were inversely related to plasma androgens in reproductively immature male white sturgeon. This study examined the relation between the putative white sturgeon hepatic cytochrome P450 3A, liver p, p'-DDE, and plasma androgens in reproductively immature male white sturgeon collected from the Columbia River, USA. Liver and plasma were collected from reproductively immature male white sturgeon commercially caught in the Columbia River. Individual levels of chlorinated pesticides and PCBs were measured in liver by GC-ECD, plasma androgens were measured by RIA, and the putative white sturgeon hepatic cytochrome P450 3A was measured in microsomes by western blotting using a polyclonal antibody generated against rainbow trout LMC5 (3A27). Of the organochlorines measured, p, p'-DDE, was detected most frequently and at the highest concentrations. The putative cytochrome P450 3A was positively correlated with p, p'-DDE. Plasma androgens were inversely correlated with p, p'-DDE and the putative cytochrome P450 3A. These data indicate the existence of a cytochrome P450 3A in white sturgeon and that, p, p'-DDE may induce the putative white sturgeon hepatic cytochrome P450 3A and reduce plasma androgen concentration *via* increased metabolism. However, additional studies are needed.

330 RELATIONSHIP BETWEEN LIVER OIL INFRARED SPECTRA AND FISH HEALTH.

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Pathophysiological problems in organisms might be derived from changes in multiparametric metabolic responses that can be monitored using spectroscopic methods. The metabolic nature of the liver makes this organ suitable to study alterations in metabolism associated with the spectroscopic features of the liver oil. The aim of this study was to test the hypothesis that fish liver oil content and its infrared (IR) spectroscopic profile is affected by environmental contamination conditions. Mugil incilis (Lisa) was chosen as a test species because it is one of the most common fish in the Atlantic coasts of Central and South America, and usually searches for food in the sediment where pollutants are concentrated. Fish samples were monthly caught in Cartagena Bay, a waterbody that receives multiple polluted effluents, and from Totumo marsh, a low contaminated site, both located at the North of Colombia, South America. After collection, fish size and length were measured and specimens examined for parasites. Liver oils were obtained by soxhlet extraction with dichloromethane and analyzed by IR spectroscopy. Measured condition indexes for fish from Cartagena were statistically lower than those obtained from the control site, and correlated inversely with the number of nematode parasites found in the liver and muscle. Livers from fish captured at Cartagena Bay had approximately 25% less oil content than those from Totumo Marsh. IR spectra were significantly different among sites particularly in the aliphatic, carbonyl and finger print region. These changes in spectroscopic properties were dependent on the sampling season. This study established that the IR spectroscopic profile of fish liver oil can be used to successfully discriminate between the different metabolic and health status of fish caught from sites with varied degree of contamination.

331 ISOLATION AND CLONING OF A MAJOR HEPATIC GLUTATHIONE S-TRANSFERASE FROM LARGEMOUTH BASS (*MICROPTERUS SALMOIDES*).

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A full-length glutathione S-transferase (GST) cDNA was obtained from largemouth bass (*Micropterus salmoides*) liver total RNA using 5' and 3' systems for rapid amplification of cDNA ends (5' RACE and 3' RACE). The full-length GST cDNA was 957 base pairs (bp) in length and contained an open reading frame of 666 bp, encoding a polypeptide of 222 amino acids. The bass liver GST exhibited high homology to a theta-like GST (GST-A) previously isolated from plaice (*Pleuronectes platessa*) with high catalytic activity towards 4-hydroxynonenal (4HNE), a cytotoxic and mutagenic breakdown product generated during the peroxidation of polyunsaturated fatty acids. Northern blotting analysis of largemouth bass liver RNA revealed strong expression of a GST-like hybridizing band of approximately 1 kb. In addition, affinity-purified bass hepatic cytosolic fractions displayed high catalytic activity toward 4HNE. In summary, the presence of a highly expressed GST isozyme in bass with extensive sequence homology to plaice GST-A is suggestive of a role of GST in protecting against oxidative damage in this freshwater species. Supported by NIH P42 ES07375.

332 ENVIRONMENTAL INDUCTION OF CYP1A- AND CYP2K1-LIKE PROTEINS IN TWO TROPICAL FISH SPECIES BY PRODUCED FORMATION WATER ON THE NORTHWEST SHELF OF AUSTRALIA.

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Little or no information is available regarding hydrocarbon exposure of tropical fish species inhabiting the waters near oil and gas platforms on the Northwest Shelf of Australia. In 1998, a pilot study was funded by Apache Energy Pty Ltd to evaluate the use of sub-lethal stress indicators in fish to assess exposure to produced formation water (PFW). Tropical fish, the Gold-spotted Trevally (*Carangoides fulvoguttatus*) and the Bar Cheeked Coral Trout (*Plectropomus maculatus*), were obtained from two sites impacted by PFW discharge, Harriet A (near field) and Harriet C (far field) and a non-impacted reference site, the Montebello Islands. Microsomal fractions from both Trevally and Coral Trout, each had one immunodetectable CYP1A- and one CYP2K1-like protein. CYP1A-like protein was expressed in a gender-specific manner with male Trevally from the reference site expressing a 3.5-fold higher level compared to reference site females. Only female Trevally had significantly higher CYP1A-like protein levels at both platform sites compared to the reference site females. Gender-specific induction of CYP2K1-like protein occurs in the Gold-spotted Trevally as PFW-exposed males express higher levels than PFW-exposed female Trevally. Both male and female Coral Trout showed increased (not significant) levels of CYP2K1-like proteins at the impacted sites as compared to the reference site. These data suggest possible PFW contamination near the platforms that produces induction of both CYP1A- and CYP2K1-like proteins. While induction of CYP1A is considered to be the most sensitive response to exposure to petroleum hydrocarbons, increases in CYP2K1-like proteins in fish have not previously been described as a consequence of environmental petroleum hydrocarbon exposure.

333 ENVIRONMENTAL RISK ASSESSMENT OF DULOXETINE HYDROCHLORIDE.

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The present work was conducted to assess environmental risk from the therapeutic use of duloxetine hydrochloride, a serotonin/norepinephrine reuptake inhibitor. Specifically, consequences of exposure to the environment *via* patient use and subsequent discharge from sewage treatment plants were evaluated. Environmental chemistry studies were conducted to determine the disposition of duloxetine hydrochloride in the environment. The water solubility of duloxetine hydrochloride is 21.6, 2.74, and 0.331 g/L at pH 4, 7, and 9, respectively. The log octanol/water partition coefficient is 0.781, 1.54, and 3.35 at pH 4, 7, and 9, respectively, suggesting that there is little potential for bioconcentration. The organic carbon sorption coefficient measured in sludge ranged from 2893 to 4296 indicating the potential for removal from the aqueous compartment *via* sorption to biosolids. Other minor depletion mechanisms include hydrolysis (half-life is 1.5 to 3.5 months) and aerobic biodegradation (no significant degradation after 8 days in simulated sewage treatment). To assess the effects of duloxetine on environmental organisms, standard toxicity tests were conducted. The 3-hr EC50 for inhibition of respiration in sewage microorganisms is 36.5 ppm duloxetine. The 96-hr LC50 of duloxetine in rainbow trout and the 48-hr EC50 in *Daphnia magna* are 1.3 ppm and 2.4 ppm, respectively. Algae were the most sensitive aquatic species in acute toxicity tests with duloxetine. The EC50 and NOEC in a 72-hr test with *Pseudokirchneriella subcapitata* were 0.064 and 0.011 ppm duloxetine, respectively. The chronic toxicity of duloxetine was determined in a full life-cycle test with *D. magna*. The most sensitive endpoint was reproduction, with EC50 and NOEC values of 0.28 and 0.011 ppm duloxetine, respectively. Based on these data and predicted environmental concentrations, the therapeutic use of duloxetine hydrochloride is not considered to pose a significant risk to the environment.

334 HOW ARE PCB CONGENERS DIFFERENT IN FILLETS OF FISHES AROUND LAKE MICHIGAN?

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How different are PCB congeners in fillets of various fishes around Lake Michigan in the last decade? DDT has adversely affected songbird reproduction. With amelioration of DDT pollution, clutch size of eggs in a bird's nest increases. PCB disrupts endocrine-system functions leading to increase in endometriosis, fetal abortion, glomerulonephropathies, osteoporosis, and a decline in the population, Helle-Bergman-Olsson's hyperadrenocorticism associated with environmental exposure to PCB/DDT". To protect the environment and human health, prevention from exposure to PCB along the food chain is of paramount importance. It is not only importan to measure PCB in the abiotic environment, but especially along the entry point into the food chain, the fish fillets. PCB concentrations in 14 different fish species (brown trout, carp, chinook salmon, coho salmon, lake trout, northern pike, rainbow smelt, rainbow trout, lake sturgeon, longnose sucker, walleye, white sucker, whitefish, yellow perch) around Lake Michigan were examined in the last decade with capillary gas chromatography and electron capture technique. It is noted that fishes, such as the carp with longer life spans and a dependence on feeding detritus accumulated more PCBs. This combined with the environmental/biological half-lives of PCB congeners led to a higher concentration and more varieties in carp in comparison with fillets of the salmon, trout and whitefish. These differences may have contributed to different problems in human health.

335 MAXIMALLY PERSISTENT PCB CONGENERS IN FISHES AND HUMANS.

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We analyzed PCB tissue burdens in humans with intermittent links to PCB contaminated fish. Pattern recognition by principal components analysis was applied to data on Ojibwa native Americans, Great Lake fishermen and non-great lake USA gamefishermen as well as PCB contaminated fish fillets determined by electron-capture-gas chromatography. Fish consumption data were obtained through questionnaires. Local-area-microenvironment PCB congeners pattern is the principal determinant factor in PCB human-contaminant-serum-patterns. Among PCB congeners, ortho position is the most frequent position regardless whether they are the gamefishermen or the Ojibwa Indians. The penta and hexachlorinated isomers are the most prevalent. The combination of these characteristics, the ortho-chlorination, the penta and hexachlorinated isomers determine not only the olubility/bioavailability, but also the bioaccumulation/ biomagnification in all organisms including fish consuming humans. Disclaimer: The conclusions and opinions in this abstract are those of the authors and do not represent affiliated institutions

336 BIOASSAY-BASED FRACTIONATION OF AN ANDROGENIC PULP MILL EFFLUENT FROM THE FENHOLLOWAY RIVER IN FLORIDA.

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Pulp mill effluent samples from the Fenholloway river in Florida were collected and tested using a bioassay-based fractionation approach, to attempt to identify the causative agent(s) responsible for observed androgenic effects in female fish from the system. Androgenic activity of the effluent and subsequent fractions was "tracked" through the process using CV-1 cells transfected with a human androgen receptor -MMTV-luciferase promoter-reporter system (Parks et al. 2001. Toxicol. Sciences. 62:257). The goal of the approach was to separate biologically-active components from non-active components of the complex effluent matrix to facilitate the identification of the chemical(s) responsible for androgenic activity. Initially organic chemicals were isolated from the effluent using a C_{18} solid phase extraction (SPE) cartridge, which was subsequently eluted with graded concentrations of methanol in water. SPE fractions which exhibited *in vitro* androgenic activity were then fractionated further using high pressure liquid chromatography (HPLC) into twenty five discrete fractions. In these studies, multiple HPLC fractionation steps were required to adequately simplify samples for chemical identification. The HPLC fractions displaying activity in the CV-1 cell assay were then analyzed using gas and liquid chromatography linked with electron impact, or chemical ionization (both positive and negative ion) mass spectrometry. Androstenedione, a steroid previously identified by other researchers as responsible for androgenicity of this effluent, was found in the effluent sample but not in the active HPLC fractions. Additional mass spectrometry results to date indicate the presence of many steroidal compounds, as well as a few halogenated chemicals in the active fractions. *This abstract does not necessarily reflect EPA policy.*

337 IDENTIFICATION OF ENVIRONMENTAL ESTROGEN-LIKE CHEMICALS IN INDUSTRIAL WASTEWATER BY GC/MS.

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Alkylphenols and phytosterols possess estrogen-like activity. Exposure to these chemicals has been implicated in the development of adverse health effects such as abnormal reproductive tract development, sterility, and cancer. Alkylphenol polyethoxylates (APEs) are used as pitch dispersants, wetting agents, and as drainage and deresinating aids in the pulp and paper industry. Alkylphenols are degradation by-products of APEs and b-sitosterol, a phytosterol, can leach out of pine trees that are used in the pulping industry. The goal of this study was to develop a gas chromatography/mass spectrometry (GC/MS) based method to detect individual estrogenic chemicals from industrial wastewater samples in a single run. Water samples were collected from an anonymous pulp mill in the state of Alabama by grab sampling in front of a discharge pipe, which transports wastewater from a settling pond to a river. The concentrated extracts from the water samples were derivatized with N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), and analyzed for trimethylsilyl (TMS) derivatives by GC/MS in select ion monitoring mode (SIM). Known estrogenic chemicals 4-nonylphenol (4-NP), 4-tert-octylphenol (4-t-OP), 4-octylphenol (4-OP), and b-sitosterol were used as standards. Detection limits for 4-t-OP, 4-NP, 4-OP, and b-sitosterol were 0.020 mg/L, 0.027 mg/L, 0.046 mg/L, and 1.25 mg/L, respectively. Sample recovery of 4-t-OP, 4-NP, and b-sitosterol was 69%. Using this method we detected 4-t-OP, 4-NP, and b-sitosterol in the effluent at concentrations of 3.45, 6.62, and 19.92 mg/L, respectively. Thus, our study indicates that this GC/MS method is highly efficient to detect environmental estrogen-like chemicals in contaminated water sources and to monitor the quality of water.

338 KINETICS AND BINDING OF FUMONISIN IN A MODEL SOIL SYSTEM.

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Fumonisin B_1 (FB_1) is a water soluble, carcinogenic mycotoxin produced by the fungus *Fusarium moniliforme*, which is parasitic to corn plants. It can be found in the ear, roots, and stalks of plants. It is estimated that 90% of the FB_1 consumed by

livestock is excreted unmetabolized. The objectives of this research are to determine 1) the kinetics of FB_1 interaction with soil constituents, and 2) if FB_1 is chemically modified in the soil. Leachate columns were used to determine the movement of FB_1 through soil matrices comprised of washed sand and 0%, 50%, 75%, or 100% Cecil sandy loam soil. The movement of FB_1 was compared to that of bromophenol blue (Bb), a dye that moved freely through the soil columns. FB_1-contaminated corn screenings or extracts containing FB_1 were placed on the surface of the soil columns. When corn screenings were used as the source of FB_1, a rain simulator was attached to the top of the column and water was allowed to saturate the corn screenings (extracting the FB_1) and percolate through the column eluting the FB_1. The 100% sand columns slightly retarded the efflux of FB_1 relative to Bb but did not appear to chemically alter the FB_1. The recovery of FB_1 decreased with increasing concentrations of Cecil sandy loam soil. At 0%, 50%, 75% and 100% Cecil sandy loam, approximately 80%, 60%, 50% and 20% of the FB_1 was recovered in the column leachate, respectively. The FB_1 retained on the 100% Cecil sandy loam column was tightly bound as evidenced by the fact it could not be extracted using acetonitrile:water (1:1). However, approximately 40% of the retained FB_1 was extractable using 5% formic acid:acetonitrile (1:1) indicating that the nature of the interaction was probably ionic. The result of this study indicate that FB_1 is probably stable in the soil environment and suggests that while some is tightly bound, under certain environmental conditions FB_1 could be released, become biologically available and possibly contaminate the water supply.

339 INCUBATION WITH TOP SOIL ENHANCES SOLUBILIZATION OF RA FROM OIL AND GAS PRODUCTION SCALE AND SLUDGE SAMPLES.

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Large quantities of formation waters (brines) are co-produced with hydrocarbons from oil and gas wells. Dissolved solutes from the brines precipitate forming scale in and on production equipment and sludge in storage tanks. Ra is one of the solutes that may precipitate rendering the scales and sludges radioactive. One method under consideration for disposal of scale and sludge wastes is land spreading in which the material is spread on the ground, tilled into the top few centimeters of soil, and then covered over with a layer of "clean" soil such that the radioactivity is below governmental action levels, and the site can be considered uncontaminated. One of the assumptions that this method of disposal is based upon is that the Ra and other inorganic components of the scales and sludges are highly insoluble, relatively immobile in the environment, and low in biological availability. To test this assumption the leaching of Ra from radioactive scale and sludge samples was examined for the scale and sludge samples alone and after mixing and incubating them under moist conditions with top soils. It was found that incubating the samples with top soils increased the leaching of Ra from them by several fold. This finding is remarkable in light of the finding that soil complexes most of the soluble Ra added to it and soils mixed with scale or sludge complex even greater proportions of the soluble Ra added to them. Even though the complexed Ra is difficult to extract from the soil it is expected to be much more biologically available and mobile in the environment than in its original insoluble form. The indication is that incubation of scales and sludges with top soils, such as with land farming, might result in significant enhancements of the environmental mobility and bioavailability of the Ra that they contain.

340 NATURAL AND ANTHROPOGENIC PROCESSES THAT CONCENTRATE MN IN RURAL AND URBAN ENVIRONMENTS OF THE LOWER MISSISSIPPI RIVER DELTA.

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Natural processes of Mn accumulation and the legacy of Pb additives in fuel for predicting Mn in the environment are the topics of this study. Data is from alluvia and sediments of the lower Mississippi River Delta and a soil metal survey of metropolitan New Orleans. The "railroad manganese," "industrial manganese" and "dynamic aquifer-stream transfer of Mn" hypotheses were tested. The results support the proposal that friction between Mn-rich steel wheels and rails contribute Mn to the environment. Sediment loads of Mn upstream and downstream from the LA industrial corridor were similar (P-value = 0.77) and this data does not support the industrial manganese hypothesis. Data medians of the alluvium surface (59 mg/kg), the aquifer (159 mg/kg) and the riverbank (513 mg/kg) where Mn-rich clay precipitates during aquifer discharge support the aquifer-stream transfer hypothesis. Soils along rural and urban roads exhibit respectively, medians of 216 and 85 mg/kg for Mn, and 17 and 212 mg/kg for Pb. The survey of metropolitan New Orleans indicates an increase from rural to interior urban communities by a factor of 4 for Mn and nearly 3 orders of magnitude for Pb. Assuming 1999 US on-road fuel use, at 8.3 mg of Mn per L, 5 thousand metric tons of Mn would be consumed. If 13% of the Mn added to fuels were emitted, 650 metric tons of Mn would become aerosols annually, and 87%, or 4, 350 metric tons would remain in the engine. The 1999 TRI for Mn shows 108 metric tons as stack releases and 370 metric tons as total emissions. In 1999, 60.5% of vehicle travel occurred in urban and 39.5% occurred in non-urban areas. Annually, Mn in fuel would emit 390 and 260 metric tons respectively in urban and non-urban areas. Mn would mimic the pattern for Pb. Release of Mn aerosols into communities currently polluted with Pb infringes on Executive Order 12898 concerning Federal redress of environmental justice in minority and low-income populations. Funded by ATSDR/MHPF cooperative agreement #U50/ATU398948 to Xavier University of LA.

341 REDUCTION OF THE TOXICOLOGIC EFFECTS OF PROLONGED HYDROCARBON VAPORS INHALATION IN CONTAMINATED BUILDINGS BY BIOREMEDIATION.

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Contamination of building structural elements which may be a result of fuel oil spills, catastrophic flooding etc., results in the release of chemicals into the air over the course of years. An efficient method of reducing the human exposure to the hydrocarbons is suggested based on bioremediation, i.e. application of live microorganisms on polluted building materials, such as wood and concrete. The purpose of this bench-scale study was to evaluate the feasibility of bioremediation for the removal of hydrocarbons using naphthalene and n-hexadecane as model pollutants. Radiolabelled hydrocarbon was applied on standard pieces of building materials followed by the adsorption of bacteria. Then pollutant degradation efficiency was monitored by scintillation counting. Two kinds of experiments were conducted: shaking the samples with biomass in mineral medium (shaking flask procedure) and attaching the building material samples on the agar medium where the biomass has been grown (overlay procedure). The observed similarity of pollutant removal kinetics for shaking flask and overlay procedures indicated that the rate-limiting step appears to be diffusion of the hydrocarbon within the pores of a building material. Moisture content of the samples had a critical influence on the degradation efficiency. Water was shown to hinder the diffusion of hydrocarbons in wood and concrete. This effect was used to accelerate the pollutant removal by adjusting the protocols for biomass application. Among the factors influencing hexadecane removal efficiency, the following conditions of biomass growth (prior to their application) were found to be critical: the time and temperature of cultivation, composition of nutrient medium, and the regime of re-inoculations. Preventing the biomass starvation resulted in a significant increase of pollutant removal. For 6-mm thick wood and one-gram concrete samples, maximum hexadecane removal achieved without applying new biomass was 85-95%, which required ca. 2 weeks.

342 MINERALOGICAL AND GEOCHEMICAL VARIATIONS AMONG ASBESTOS STANDARDS.

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It is well known that exposure to asbestiform mineral dust is associated with lung diseases such as asbestosis, malignant mesothelioma, and bronchogenic carcinoma. Past attention primarily focused on human exposure and health effects of commercial asbestos. In recent years, there has been renewed recognition of the potential deleterious health effects of asbestos that occur as accessory minerals in rocks (i.e.;serpentinite, a common rock in US) and in some mineral commodities used commercially (i.e.;vermiculite, talc). Within the past decades, many *in vitro* and *in vivo* studies have shown asbestos to be cytotoxic and mutagenic, yet the mechanisms responsible are not fully understood. These questions can be best answered through continued and increased collaborative research between the earth science and health-related communities. One example of this is the mineralogical and geochemical characterization of individual asbestos family members. The USGS has assembled 5 sets of asbestos standards, which have been previously used in toxicological studies (Standard sets:5 amosites, 4 anthophyllites, 6 chrysotiles, 5 crocidolites, 4 tremolites). Presently, we have identified the mineralogical and geochemical characteristics for each set of asbestos standards. Chemical analyses indicate that elemental (i.e.;Al, Ca, Co, Cr, Fe, Ga, Li, Mg, Mn, Ni) content varies between the different asbestos minerals. Variations within the standards of the same asbestos mineral sets are present. X-ray diffraction and SEM also indicate differences in asbestos habit (crystal shape, cleavage pattern) and the presence of other minerals as contaminants between different asbestos standards and within the sets of asbestos standards. These variations between the asbestos standards or those within the sets

of the same asbestiform mineral may be responsible for conflicting toxicological results, ultimately inhibiting mechanistic identification. Toxicity studies utilizing each group of standards are in progress.

343 DILUTED DIESEL EXHAUST EMISSIONS DO NOT INDUCE CHANGES IN PERIPHERAL BLOOD MICRONUCLEI LEVELS IN SUBCHRONICALLY EXPOSED MALE AND FEMALE A/J MICE.

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Focus has been directed toward the cancer risk associated with diesel emissions' contribution to air pollution in both occupational and environmental settings. As one component of the National Environmental Respiratory Center's long-term goal to assess the carcinogenic potential of many anthropogenic source emissions, we determined the clastogenic ability of subchronic exposures of diesel emissions to induce peripheral blood micronuclei in the strain A/J mouse. One hundred male and one hundred female A/J mice (10 male and 10 female mice per exposure concentration) were exposed (whole body, Hazelton H2000 chamber) to log doses of diesel emissions generated from one of two 2000 Cummins B series engines operated on the heavy duty test cycle with EPA certification diesel fuel. Chamber concentrations were determined by diesel exhaust particulate matter and maintained at 0, 30, 100, 300 and 1000 $\mu g/m^3$ respectively. Exposures were conducted for 6h/day, 7 days a week, for a period of six-months. Micronuclei were assessed in heparinized peripheral blood from each animal by flow cytometry (~20, 000 reticulocytes counted per animal). Levels of micronuclei in clean air-exposed, 6-month old mice were consistent with previously published reports in juvenile and age-matched A/J mice (within 3 standard deviation of the mean). As analyzed by one-way ANOVA and the Student t test for pair wise comparison to control, there were no differences between the mean levels of micronuclei in control and exposed mice ($P \leqq 05$). These findings indicate that exposure of A/J mice to diesel emissions diluted up to $1000 \mu g/m^3$ does not induce clastogenic changes in micronuclei levels as measured in peripheral blood. Research conducted by the National Environmental Respiratory Center (NERC) with support from multiple government and industry sponsors, including the USEPA. This abstract is not intended to represent the views or policies of any NERC sponsor

344 USING THE MOUSE LYMPHOMA TK ASSAY TO COMPARE THE MUTAGENICITY OF MAINSTREAM SMOKE CONDENSATE FROM RESEARCH CIGARETTES.

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The mouse lymphoma TK assay - microtiter version - has been established and optimized to quantitatively determine the *in vitro* genotoxicity of mainstream smoke condensate (MSC) in order to complement our existing testing battery. The assay was validated with the positive control substances methyl methanesulfonate and benzo(a)pyrene and MSC from the Reference Cigarette 1R4F with and without a metabolic activation consisting of the postmitochondrial (S9) fraction of the livers from rats treated with Aroclor 1254. Reproducible responses - with a coefficient of variation of less than 20% - were found for the positive control substances and for the 1R4F-MSC. To test whether the assay is able to discriminate different cigarette types, MSC from five research cigarettes containing different tobacco blends was prepared and assayed. Positive responses were obtained for all cigarettes, with concentration-related increases in the mutant frequency. To compare the mutagenicity of the cigarettes, the dose-response curve for the mutant frequency was calculated by nonlinear regression analysis. From each dose-response curve, the effective concentration for a 3-fold increase over the spontaneous mutant frequency was calculated and compared. The comparison showed that without S9, the activity of MSC from a 100% Burley blend cigarette was lower than that of the other blends; with S9 a similar trend was seen. This is different from the results seen in the S. typhimurium reverse mutation assay in strains TA98 and TA100 with S9, where the Burley cigarette was more active than the 100% Bright blend cigarette. This supports the need for a genotoxicity testing battery of complementary assays for the comprehensive testing of cigarette smoke. INBIFO is a Philip Morris research laboratory.

345 GENOTOXICITY OF NICKEL-CONTAINING SAMPLES IN 10T1/2 MOUSE EMBRYO CELLS: PREDICTIONS OF CARCINOGENIC POTENTIALS AND IMPLICATIONS FOR MECHANISMS OF CARCINOGENESIS.

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Long-term inhalation exposures of workers to mixtures of some insoluble and soluble nickel compounds in specific nickel refinery operations in the past were associated with excess lung and nasal cancer. For example, nickel subsulfide induced tumors in rats by inhalation. Animal data by relevant routes of exposure are not available for all nickel compounds, metallic nickel, or nickel-containing alloys. *in vitro* studies can directly evaluate genotoxicities of new nickel-containing substances relative to nickel compounds that are well-characterized in animal studies. We therefore determined relative genotoxicities of nickel-containing samples in short-term *in vitro* assays, and used these relative genotoxicities to rank samples for their predicted carcinogenic potentials and prioritize samples for *in vivo* testing. Previously, we have shown that two nickel subsulfide samples (< 10μm diameter) induced strong, dose-dependent morphological transformation in 10T1/2 cells. Spherical particles of elemental nickel samples (ENi-1, average diameter of 2.3 μm and ENi-3-7 average diameter 11 μm) and two stainless steel samples (3.5 μm and 8.5 μm average diameter) did not induce morphological transformation. The order of cytotoxic potency of a new set of samples (LC_{50} values in parentheses) was: Ni_3S_2 (1.35 μm)(0.35 ± 0.2 μg/ml) > Ni (UF) (0.45 ± 0.3 μg/ml) > $Ni(OH)_3$ (powdered form) (2.96 ± 2.4 μg/ml) > $NiCO_3$ (4.08 ± 3.8 μg/ml) >> SS (<3.0 μm) (455 ± 26.1 μg/ml). Particles of stainless steel < 3.0 μm were not taken into and did not induce morphological transformation in, 10T1/2 cells. Small particles of nickel subsulfide (Ni_3S_2, 1.35μm) were phagocytosed in a dose-dependent manner (67.7% of the cells at 1.0 μg/ml). Spherical particles of ultrafine metallic nickel (UF) were phagocytosed (60.5% of cells at 5.0 μg/ml), induced chromosome breakage and morphological transformation.

346 OXIDANT-INDUCED DNA DAMAGE BY QUARTZ AND HYDROGEN PEROXIDE IN ALVEOLAR EPITHELIAL CELLS.

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Studies with respirable quartz using naked DNA as a target suggest that hydroxyl-radical formation by the particle itself may play a role in the DNA-damaging properties of quartz. However, it is not known whether this pathway is important for DNA damage in alveolar epithelial cells which are considered as target cells for quartz carcinogenesis. Therefore, we determined DNA damage by quartz or hydrogen peroxide in A549 human and RLE rat alveolar epithelial cells using the alkaline comet-assay. The hydroxyl radical generation capacity of the quartz was analysed by electron spin resonance (ESR), and by immunocytochemical analysis of the hydroxyl radical-specific DNA lesion 8-hydroxydeoxy-guanosine (8-OHdG) in the epithelial cells.Quartz particles as well as hydrogen peroxide were found to cause a dose-dependent increase in DNA strand breaks in both cell lines. DNA damage by quartz was significantly reduced in the presence of the hydroxyl-radical scavengers mannitol or DMSO. The involvement of hydroxyl radical was also demonstrated by ESR measurements, and by the ability of the quartz to induce formation of 8-OHdG in both cell types. In conclusion, our data show that quartz elicits DNA damage in human and rat alveolar epithelial cells, and indicate that these effect are driven by intrinsic hydroxyl-radical generating properties of the particles.

347 AGE, GENDER, AND SPECIES DEPENDENT MUTAGENICITY IN T-LYMPHOCYTES FROM MICE AND RATS EXPOSED TO 1, 3-BUTADIENE BY INHALATION.

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To determine the effects of age, gender, and species on the mutagenicity of 1, 3-butadiene (BD), male and female B6C3F1 mice and F344 rats (4-5 or 8 week-old) were exposed to 0 or 1250 ppm of BD for 2 weeks by inhalation (6 h/day, 5 day/week). The mutation manifestation curves were defined by measuring the Hprt mutant frequencies (Mfs) in splenic T-lymphocytes at multiple time points after BD exposure using a T-cell cloning assay, and the mutagenic potencies of BD were calculated as the differences of area under the manifestation curves of treated *versus* control animals. The mutagenic potency of BD in 4-5 week-old female mice (69.6, with a 95% confidence interval of 61.1 and 78.7) was not significantly different from that in 8 week-old female mice (57.0, with a 95% confidence interval of 44.7 and 71.5); but, the maximum BD-induced Mfs were observed at 5 weeks post exposure in younger mice *versus* 8.5 week post exposure in older mice, with the peak values in BD-induced Mfs in 4-5 week old mice ($17.8 \pm 6.1 \times 10^{-6}$) being 2.2–fold greater than that in 8 week-old mice ($8.0 \pm 1.0 \times 10^{-6}$). Thus, while shape of the mutant T-cell manifestation curves for spleens of BD-exposed 4-5 week-old and 8 week-old mice were different, the mutagenic burden was the same for both age groups. BD-induced Hprt Mfs in female mice ($17.8 \pm 6.1 \times 10^{-6}$) were 2.3-fold greater than those in male mice ($7.7 \pm 3.3 \times 10^{-6}$) at 5 weeks post exposure, while BD-induced Hprt Mfs in female rats ($7.2 \pm 5.5 \times 10^{-6}$) were 1.9-fold greater than those in male rats ($3.9 \pm 2.8 \times 10^{-6}$) at 4 weeks post exposure. The results of these

species and gender evaluations demonstrate that the overall pattern for mutagenic susceptibility in BD-exposed rodents is female mice > male mice > female rats > male rats, consistent with the current knowledge of species and gender differences in metabolism and carcinogenic susceptibility.

348 NONHOMOLOGOUS END JOINING IS NOT A SIGNIFICANT PATHWAY FOR REPAIR OF BENZENE-INDUCED DNA DOUBLE STRAND BREAKS.

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Benzene is a genotoxic carcinogen that induces chromosomal breaks as a primary mode of genotoxicity in the bone marrow (BM). We showed that chronic exposure to inhaled benzene induced a p53-regulated DNA damage response in mouse BM. We investigated the nonhomologous end joining (NHEJ) pathway of DNA double strand break (DSB) repair in benzene genotoxicity. Quantitative RT-PCR was used to determine Ku80 mRNA levels in the BM of C57BL/6 mice exposed to inhaled benzene (100 ppm) for 15 wk., hypothesizing that benzene would induce expression of genes, such as Ku80, involved in NHEJ. However, Ku80 mRNA levels in benzene-exposed mice were not significantly altered. Next, we compared the genotoxic effects of inhaled benzene on C57BL/6 and 129/SvJ mice which differ at the *Prkdc* locus encoding the catalytic subunit of DNA-PK (DNA-PKcs) that binds to the Ku70/Ku80 heterodimer during NHEJ. C57BL/6 mice have a wild-type allele at *Prkdc* while 129/SvJ mice have a *Prkdc* functional polymorphism that leads to diminished DNA-PK activity and enhanced apoptosis in response to radiation-induced damage. We expected mice with deficient DNA-PK activity to be more susceptible to the genotoxic effects of inhaled benzene. Mice were exposed to 0, 10 or 100 ppm benzene for 6 h/day, 5 days/week for 2 weeks. Both strains had decreased WBC and lymphocyte counts in the blood with increasing dose of benzene. Flow cytometric cell cycle analysis on BM cells stained with propidium iodide revealed no G1 arrest in either strain at any dose of benzene. Genotoxicity was assessed by a flow cytometry assay that distinguishes micronucleated mature normochromatic erythrocytes (MN-NCE) from micronucleated reticulocytes (MN-RET) in blood. We observed comparable increases in MN-NCE and MN-RET populations in both C57BL/6 and 129/SvJ mice following benzene exposure, suggesting the difference in *Prkdc* alleles does not affect benzene-induced genotoxicity. The data indicate that NHEJ, mediated by the Ku heterodimer and DNA-PKcs, is not a significant pathway in the repair of benzene-induced DSB.

349 GENETIC SUSCEPTIBILITY TO BENZENE-INDUCED TOXICITY: ROLE OF NAD(P)H:QUINONE OXIDOREDUCTASE-1 (NQO1) AND MICROSOMAL EPOXIDE HYDROLASE (mEH).

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Chronic benzene is hematotoxic, genotoxic, and carcinogenic in humans and rodents, causing a variety of hematopoietic disorders. The enzyme systems involved in activation and detoxication reactions of benzene metabolism are likely genetic determinants of individual variability and risk in response to benzene. NQO1 catalyzes the detoxication of 1, 4-benzoquinone, proposed as an ultimate toxic benzene metabolite. mEH metabolizes benzene oxide to dihydrodiol, which can then be converted to catechol or trans, trans-muconaldehyde, both of which can lead to genotoxic metabolites. Our hypothesis is that the NQO1 and mEH pathways are critical to benzene-induced toxicity. We exposed NQO1-/- and mEH-/- mice to inhaled benzene (10 or 100 ppm) for 2 weeks and then determined the hematotoxicity and genotoxicity relative to the 129/SvJ (wild-type;WT) mice. Micronucleated (MN) polychromatic erthyrocytes (PCE; immature RBC) assessed manually in the bone marrow and by flow cytometry in the blood were used as a measure of genotoxicity. In preliminary studies, WT mice were almost twice as sensitive to benzene-induced genotoxicity (2.7% MN-PCE, $p<0.01$) compared to NQO1-/- mice (1.3% MN-PCE, $p<0.01$). mEH-/- mice were not sensitive to benzene (0.3% MN-PCE) compared to WT mice (3.5% MN-PCE, $p<0.01$). p21 mRNA gene expression measured by quantitative RT-PCR in bone marrow was used as a genotoxic biomarker of the p53 DNA damage response pathway. WT and NQO1-/- mice had a >7-fold increase at the 100 ppm dose over unexposed controls in p21 expression in response to benzene compared to a 1.4-fold in mEH-/- mice. p21 induction in WT and NQO1-/- mice suggests the p53 pathway is activated. These preliminary results suggest that NQO1 deficiency does not lead to increased benzene-induced genotoxicity and that mEH deficiency results in protection against benzene-induced genotoxicity.

350 INDUCTION OF MICRONUCLEI BY PHENOL IN MOUSE BONE MARROW IS ASSOCIATED WITH TREATMENT-INDUCED HYPOTHERMIA.

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Asanami and coworkers have reported an association between hypothermia and micronuclei (MN) formation in mice (Mutat. Res. 413, 1997). Since previous studies have shown weak positive responses in the mouse micronucleus test (MNT) following intraperitoneal (i.p.) doses of phenol at or near the LD50, the current study evaluated the capability of phenol to cause hypothermia in mice and the association of MN formation and phenol-induced hypothermia. CD1 mice received a single i.p. dose of a vehicle control or phenol ranging from 0–500 mg/kg. Body temperatures (BT) of all mice were monitored at baseline and at multiple times post-dosing using subcutaneous digital transponders. Phenol at 300 mg/kg caused a significant and prolonged hypothermia in mice (up to 7°C drop in BT). BT were not significantly altered at doses below 300 mg/kg, while lowered BT and marked lethality occurred at 400 & 500 mg/kg. In the MNT, male and female CD1 mice (0, 30, 100 or 300 mg/kg phenol, single dose, i.p.) and a positive control group (cyclophosphamide (CP), 120 mg/kg p.o.) were evaluated for MN formation at 24 & 48 hr. BT were monitored in phenol-treated animals as described above. Treatment with CP induced a significant increase in MN formation. Phenol at 300 mg/kg induced a significant and prolonged hypothermia in both male and female mice as well as increases in the MN formation in bone marrow harvested at either 24 or 48 hours post-dosing. Data available to date indicate that neither BT nor MN values were affected at the lower doses of phenol in male mice. It thus appears that the induction of MN by phenol is a threshold phenomenon and the induction of MN at the maximum tolerated doses may be causally related to the induction of hypothermia. Sponsored by the American Chemistry Council's Phenol Panel, Arlington, VA.

351 CHARACTERIZING MUTATIONS INDUCED BY BENZIDINE AND BENZIDINE-RELATED COMPOUNDS USING BASE-SPECIFIC SALMONELLA TESTER STRAINS AND REDUCTIVE METABOLISM.

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Benzidine (Bz), 4-aminobiphenyl (ABP), 3, 3'-dichlorobenzidine HCl (DCBz), 3, 3'-dimethylbenzidine (DMBz), 3, 3'-dimethoxybenzidine (DMOBz) and the benzidine congener-based dye trypan blue (TB) produce primarily frameshift mutations in Salmonella typhimurium. However, the base-substitution strain TA100 also responds to these compounds when S9 is present. DNA sequence analysis by other investigators shows that ABP induces frameshift, base-pair, and complex mutations. Also, an uninduced hamster liver S9 preparation with glucose-6-phosphate dehydrogenase, FMN, NADH and 4 times glucose 6-phosphate gives a stronger mutagenic response than the conventional plate incorporation with a rat S9 activation mixture for all the compounds tested. Using the base-specific tester strains of S. typhimurium (TA7001 - TA7006) with the above reductive metabolic activation system, we surveyed these compounds for the ability to produce specific base-pair substitutions after reductive metabolism. Bz was weakly mutagenic in TA7005 (0.04 revertants/μg). ABP was mutagenic in TA7002 (1.4 revertants/μg), TA7004 (0.6 revertants/μg), TA7005 (2.98 revertants/μg), and TA7006 (0.4 revertants/μg). DCBz was weakly mutagenic in TA7004 (0.01 revertants/μg). It was concluded that benzidine induced some CG–>AT transversions in addition to frameshift mutations. ABP induced TA–>AT, CG–>AT, and CG–>GC transversions as well as GC–>AT transitions. DCBz induced only GC–>AT transitions. Because DMBz, DMOBz and TB were not mutagenic in this base-substitution mutagen detection system, their mutagenic activity was attributed strictly to frameshift mechanisms. This is an abstract of a proposed presentation and does not necessarily represent the views of the USEPA.

352 EVALUATION OF THE GENOTOXIC POTENTIAL OF POLYVINYL ALCOHOL IN A SHORT-TERM BATTERY OF TESTS.

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Polyvinyl Alcohol (PVA) is a water soluble synthetic polymer prepared from the polymerization of vinyl acetate and the subsequent hydrolysis of polyvinyl acetate. PVA is used as a polymer for film coating pharmaceutical and dietary supplement

tablets where moisture barrier/protection properties are required. A special grade of PVA was used for these studies that is produced to meet global pharmaceutical standards using appropriate good manufacturing practices (GMP's). The PVA grade is characterized by both compendial and proprietary specifications. The impurities are strictly monitored by process controls, stringent specifications and analytical methods developed by the manufacturer. PVA was tested in a battery of short-term genotoxicity assays. A bacterial reverse mutation assay was conducted using S. typhimurium strains TA98, TA100, TA1535, TA1537 and E.coli strain WP2 uvrA in the presence and absence of Arochlor-induced rat liver S9. PVA, when tested in water up to 5000 µg/plate, showed no evidence of mutagenicity in these assays. An *in vitro* mammalian cell mutation assay based on detection and quantitation of a forward mutation in a subline of mouse lymphoma L51789Y cells at the thymidine kinase locus was conducted. PVA did not demonstrate mutagenic potential in this assay at concentrations up to 5000 µg/mL. In a bone marrow micronucleus test for cytogenetic damage using male and female mice, PVA was administered orally by intragastric gavage at doses of 500, 1000 and 2000 mg/kg/day. PVA did not show any evidence of causing chromosome damage or bone marrow toxicity at doses up to 2000 mg/kg/day. Results from these studies indicate that PVA was devoid of any genotoxic potential based on a standard genotoxicity testing battery.

353 INDUCTION OF DNA DAMAGE AND REPAIR BY THE MYCOTOXIN OCHRATOXIN A IN DIFFERENT CELL TYPES.

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In domestic and laboratory animals the mycotoxin Ochratoxin A (OTA) is nephrotoxic, immunosuppressive, and carcinogenic. Additionally, it is suspected to be responsible for urinary tract tumours in patients suffer from Balkan Endemic Nephropathy. In this study the induction of DNA damage by OTA and the subsequent DNA repair was investigated with the Comet Assay. Several cell types with different metabolic competence were used: primary cultured porcine urinary bladder epithelial cells (PUBEC), Madin-Darby canine kidney cells (MDCK), and the human urothelial cell line 5637. The experimental protocol was varied using different incubation times for OTA in the presence and absence of an external metabolizing system (S9) and by using inhibitors of DNA repair (cytosine arabinose araC, and hydroxyurea HU). In MDCK and 5637 cells OTA induced single strand breaks (ssb) in a concentration dependent manner, while PUBEC cells showed weaker effects. When S9-mix was added, the genotoxic effect was significantly stronger in all cells. When DNA repair was inhibited the tail length increased dramatically and all treated cells showed ssb. A further culture of the damaged cells in the absence of any supplement resulted in a complete repair of the DNA within two hours. These results demonstrate that OTA induce ssb *in vitro* and that the damaged DNA can be repaired more effectively in PUBECs compared to cells of celllines (MDCK, 5637).

354 GENOTOXIC EFFECTS OF SOY ISOFLAVONES AND TWO MAJOR OXIDATIVE METABOLITIES.

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The isoflavonoid phytoestrogens daidzein (DAI), genistein (GEN) and glycitein (GLY) are major dietary components of soybeans. There is a growing scientific interest in phytoestrogens due to their potential beneficial properties for human health. Isoflavonoid intake seems to be inversely associated with the incidence of hormone-dependent diseases, e.g. breast cancer. Recently, the oxidative metabolism of DAI and GEN was investigated in our Lab.. Both isoflavones are converted by cytochrome P450 enzymes to several catechol metabolites, e.g. 6, 7, 4'- and 7, 3', 4'-trihydroxyisoflavone (6, 7, 4'-IF, 7, 3', 4'-IF), which may represent a metabolic activation process. Using the comet assay, we have investigated the potential of these compounds which may cause DNA damage. The extent of DNA strand breaks (SB) and oxidative DNA modifications (recognized by the purified bacterial repair enzyme formamidopyrimidine-DNA glycosylase, Fpg) was assessed in HeLa-S3 cells. As expected from results of the literature DAI does not cause any DNA damage in contrast to GEN, which leads to high amounts of SB. GLY induces a small number of SB. 6, 7, 4'-IF and 7, 3', 4'-IF cause small amounts of SB with a maximum after 2 h incubation time (as GEN and GLY) and high amounts of Fpg-sensitive sites at the end of incubation (24 h). The DNA-damaging potential of GEN is probably associated - as described in the literature - with the inhibition of topoisomerase II. 6, 7, 4'-IF and 7, 3', 4'-IF cause oxidative DNA damage and SB probably by generating reactive oxygen species *via* "redox cycling" and forming DNA adducts, respectively. Supported by Deutsche Forschungsgemeinschaft (Grant Ku 1079/5-1).

355 GENETIC TOXICOLOGY PROFILE OF INACTINE PEN110.

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The V. I. Technologies process for the purification of blood involves removal of lymphocytes followed by inactivation of viruses, bacteria, protozoa and residual lymphocytes with the compound PEN110. PEN110 achieves pathogen inactivation by modification of nucleic acid bases to disrupt replication of the genome by polymerase enzymes. During incubation of red blood cells, PEN110 has the ability to inactivate free, cell associated, and integrated HIV. Red blood cells are then extensively washed to remove PEN110 and plasma proteins. The genetic toxicology profile studies presented here are a key segment of the work to assure that exposures to any residual PEN110 will be without adverse effects. PEN110 is positive without metabolic activation in the rat hepatocyte *in vitro* unscheduled DNA synthesis (UDS) assay, in Ames base pair substitution strains TA1535 and TA100, and in the mouse lymphoma assay. The spectrum of genotoxic activity of PEN110 is, however, narrow, apparently producing only base pair mutations and showing no activity in chromosomal assays or the whole animal. PEN110 was negative in the *in vivo-in vitro* UDS assay, in the Ames frame shift strains TA98, TA1537 and WP2uvrA, in the chromosomal aberration assay in CHO cells, and in inducing chromosomal aberrations in freshly drawn human blood peripheral lymphocytes. No response was seen in the *in vivo-in vitro* UDS assay by i.v. administration at a dose giving an initial nominal blood concentration of over 250 times that which had been demonstrated to respond in culture. This indicates the presence of a rapid, effective detoxification pathway that clears PEN110 before it can modify the liver DNA. PEN110 was negative in the *in vivo-in vitro* UDS assay at a dose approximately 12, 000 fold over the anticipated human dose following a 1 unit transfusion. These quantitative genetic toxicology studies indicate that the trace amount of residual PEN110 in purified blood products represents an insignificant risk to the patient.

356 THE INDUCTION OF FRAMESHIFT AND BASE-SUBSTITUTION MUTATIONS BY EPIRUBICIN AND IDARUBICIN IN *SALMONELLA TYPHIMURIUM*.

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Clinical use of the natural anthracyclines (daunomycin and adriamycin) in chemotherapy has been limited due to cardiotoxicity and acute myleosuppression. Epirubicin and idarubicin are two recently developed anthracyclines that are used in cancer treatments. In spite of intensive investigations, the events leading to cell death and differential cytotoxicity in the presence of these antineoplastic compounds are not totally understood. A great many short-term tests have been developed that allow compounds to be screened for potential gentotoxicity. The first such assay to be optimized for convenience and sensitivity is called the *Salmonella* Mutagenicity Test. Previous studies have shown that daunomycin and adriamycin can induce frameshift and base-substitution transition mutations in prokaryotic and eukaryotic cells. While daunomycin can induce GC to AT and AT to GC events, adriamycin induces only GC to AT mutations. In this study, we show that both epirubicin and idarubicin can also induce frameshift (approximately 15 to 20 fold) and base-substitution transition mutations (approximately 3 to 8 fold) in *Salmonella*. Thus, it appears that these two compounds, like daunomycin and adriamycin, can induce a wide array of mutational events in the cell.

357 THE POTENTIAL GENOTOXICITY OF BRIVUDIN.

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Brivudin [(E)-5-(2-Bromovinyl)-2'-deoxyuridine, BVDU] is a nucleoside analogue with selective activity against varicella zoster virus (VZV) and herpes simplex virus type 1 (HSV-1). It has recently received marketing approval in Germany for the early treatment of VZV infections. Brivudin is selectively phosphorylated to the mono- and diphosphate by viral enzymes. This leads to accumulation of brivudin within virus infected cells. The diphosphate is further phosphorylated to the triphosphate by cellular enzymes. The triphosphate interacts with viral DNA-polymerase acting either as a substrate for or an inhibitor of the enzyme. Although no brivudin incorporation into non-virus infected cells has been detected, the mechanism of anti-viral action does suggest scope for some mutagenic or clastogenic potential. A comprehensive battery of *in vitro* and *in vivo* genotoxicity studies has been performed. Data are presented from the following *in vitro* assays: a *Salmonella typhimurium* reverse mutation (Ames) test; an HGPRT test in CHO cells; two chromosome aberration studies in human lymphocytes and an unscheduled DNA synthesis (UDS) study. Brivudin was consistently negative for gene mutation and did not induce UDS. Clear increases in the frequency of chromosome aberrations were seen in human lymphocytes after 44 hours of continuous treatment in the absence of metabolic activation only. Data are also presented from the following *in*

vivo assays: a UDS assay in the rat (this also included assessment of replicative DNA synthesis); two micronucleus studies in mice and a spot test in mice. These assays were all negative for genotoxicity, although a treatment related increase was seen in replicative DNA synthesis. The data indicate that brivudin has no potential for mutagenic activity. The increase in chromosome aberrations is concluded to be of no biological significance for the human safety assessment of brivudin. In addition, the data support the argument that a non-genotoxic mechanism is responsible for the reported induction of liver tumours in rats, following oral administration of brivudin for 1 year (Semler, *et al*, 1987. *J Am Col Toxicol* **6**, 557).

358 DEVELOPMENT OF INDICATOR CELLS FOR DETECTION OF GENOTOXIC COMPOUNDS: EXPLOITATION OF THE CELLULAR p53 RESPONSE.

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Our laboratory has previously developed a mammalian, *in vitro* assay for genotoxins based on the ability of cells to increase their level of p53 in response to DNA damage. Cultured cells are treated with the test substance, and at defined times following treatment, are harvested and lysed. The lysates are then analyzed for p53 by ELISA. An increase in cellular p53 following treatment is interpreted as evidence for DNA damage, and a lack of such an increase is interpreted as evidence of no significant DNA damage. To determine whether this system would work in cultured human cell lines, as well as in mouse lines, we tested several human cell lines known to express wild-type p53 for their ability to increase their p53 levels following DNA damage. Five such cell lines, U87, WI38, ZR 75-1, MCF-7 and U2OS were identified. To increase the speed and economy of this assay and to improve its ability to function in a high throughput application, we then developed indicator cells that can identify DNA damage without the need for cell lysis. Plasmids coding for the p53-responsive element fused to the gene coding for the reporter protein secreted alkaline phosphatase (SEAP) were stably transfected into human U2OS cells. Several individual clones were then isolated and characterized. When treated with the genotoxin mitomycin C, these cells increased both their intracellular level of p53 and the level of SEAP by several-fold, confirming their usefulness as indicator cells.

359 THE EVALUATION OF AGE-DEPENDENT DNA DAMAGE IN RATS BY A COMET ASSAY.

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Various environmental factors can be a cause of DNA damage, and accordingly it is conceivable the damage is accumulated with age particularly in the organs exerting high function in the body, i.e. the liver and kidney. This study examined the correlation between aging and DNA damage in the rat kidney. In addition, the vulnerability of DNA to a chemical carcinogen in the kidney was compared between young and aged rats. The detection of DNA damage was conducted with a comet assay (single cell gel electrophoresis). Male F344 rats at 9 weeks and 9 months old were treated with a vehicle (0.5% carboxymethylcellulose sodium: CMC) or chemical carcinogen (methyl methanesulfonate: MMS at 80 mg/kg). Three hours after dosing, the kidney was homogenized to make cell suspension and specimens were prepared. The specimens were electrophoresed under alkaline conditions for lysing and unwinding DNA and then stained with ethidium bromide. Fifty nuclei from one specimen per animal were observed under a fluorescence microscope to measure DNA migration as an index of DNA damage. The average values of DNA migration were statistically compared. In the vehicle control groups, DNA migration in the 9-month-old rats was 16.73 ± 1.80 μm which was statistically greater than the DNA migration (7.56 ± 0.74 μm) in the 9-week-old rats. In the MMS-treated groups, DNA migration in the 9-week-old rats was 29.46 ± 3.28 μm, whereas DNA migration in 9-month-old rats could not be determined due to severe DNA damage. The comet image in the MMS-treated group at 9 months old was similar to a typical image of apoptosis. The present study suggests that spontaneous DNA damage and vulnerability of DNA to a chemical carcinogen in the rat kidney increase with age. The higher vulnerability in the 9-month-old rats may be attributable to an age-dependent increase in accumulated oxidative damage of DNA.

360 MUTATGENICITY EVALUATION OF BIOPHARMACEUTICALS USING AN AMES-MODIFIED METHOD BY DENATURING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (DHPLC) (WAVE).

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The Ames Test evaluates the mutagenic potential of test chemicals by analyzing their effect on histidine (his) requiring strains of Salmonella typhimurium. Salmonella strain TA102, which causes a base substitution mutation, has its his gene located on a multicopy plasmid, pAQ1. After strain TA102 was exposed to the oxidative mutagen, ethylene oxide, pAQ1 was isolated (Miniprep kit, Quiagen) and the his gene was extracted from the plasmid by restriction digest(Stratagene). The restriction fragments containing the his gene were analyzed by denaturing HPLC (Transgenomic WAVE system), and mutations were detected based on the ability to visualize the formation of heteroduplexes. When a sample contains one uniform sequence of dsDNA, a single molecule is formed as the two complementary strands anneal (homoduplex). However, a sample containing two dsDNA molecules that differ by as little as a single nucleotide, can result in the formation of two homoduplexes and two heteroduplexes. Denaturing HPLC separates these molecules with the heteroduplexes eluting first. If both samples are identical in sequence, no heteroduplex will form and only one peak will be detected. Analysis of the digests of the pAQ1 plasmid by electrophoresis indicated that after 1h, 37oC, the 322bp fragment could be detected. Samples from each of the mutants were mixed with the wild type at a ratio of 1:1 and incubated at 95oC for 4 min followed by a cooling period at 25oC for 45 min. The fragments were subjected to a temperature titration (50-62oC) and were analyzed on the WAVE system. The wild-type fragments (homoduplexes) as well as the mutant fragments (heteroduplexes) were detected. Both the hetero-and homoduplexes eluted at the predicted time based on the method for mutation detection using the WAVE software. These modified Ames assay can be used to detect mutagens using a high throughput, accurate and inexpensive procedure. Evaluation of other Salmonella strains, genomic DNA and PCR products are studies which are currently in progress.

361 COMPARISON OF THE REGULATORY AMES TEST WITH THE SOS/UMU ASSAY AS PART OF LEAD OPTIMISATION SCREENING.

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Regulatory genetic toxicology produces 20-25% *in vitro* positive results per year. We compared the SOS-umu assay as a mutagenicity screen with the corresponding Ames result. The umu assay uses S.typhimurium strain TA1535/pSK1002 in which lac-Z is under the control of the SOS repair genes. Compounds were tested up to 1 mg/ml (approximately equal to the maximum dose in the Ames test, 5 mg/plate). The Ames test was performed using standard regulatory methods with S.typhimurium strains TA1535, TA1537, TA98 and TA100 and E.coli strain CM891. A number of mutagens (Aflatoxin B1, 4-nitroquinoline oxide, methyl methane sulphonate, 2-aminoantharcene, nitrofurantoin, BENZO(A)PYRENE, methyl nitro-nitrosoguanidine, daunamycin, 2-aminofluorene, 9-aminoacridine, 2-nitrofluorene and sodium azide) were tested along with non-mutagenic toxic compounds (DMSO, ampicillin, tetracycline, amphotericin B, pen/strep and gentamycin), together with 12 proprietary compounds concluded as regulatory Ames mutagens. All were tested in the presence and absence of rat liver S9 mix. The data correlated well with the Ames test, with all 12 of the classic mutagens tested were detected and no false positives found. Of the 12 proprietary compounds the umu assay detected 9. Those that were not detected were weakly positive in the Ames test and two of them in CM891 which will detect certain types of mutation that do not induce the SOS repair pathway. On the whole the umu ranking correlated well with that for the Ames test. However, one anomaly was a proprietary compound that was weakly positive in the Ames test with CM891 but strongly positive in the umu assay. There did not appear to be any difference in sensitivity of the umu assay between frameshift and base pair substitution mutations. It is concluded that it is possible screen out the majority of genotoxins at a very early stage in development. This will give vital information, which if integrated with data from other lead optimisation screens will improve the process of lead candidate selection.

362 AUTOMATION AND VALIDATION OF THE YEAST DEL ASSAY THAT DETECTS CARCINOGENS.

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DEL assays in yeast, human cells and *in vivo* in mice quantify potentially carcinogenic DNA deletion events and detect a wide variety of carcinogens, many of which are negative in other short-term genotoxicity tests. The yeast DEL assay also differentiates correctly between carcinogen / non-carcinogen structural analogs. Of 62 chemicals of known carcinogenicity tested, the yeast DEL assay correctly identified 85%. In comparison, the widely used Salmonella reverse mutation assay correctly classified only 33%. The yeast DEL assay is under development as an automated robotic screen. We have adapted the assay to a liquid, microtiter plate format using optical density growth detection. Pilot studies, focused on the parameters of cell density and growth kinetics in microtiter plates showed the feasibility of such a format. Refinement and further development allowed the detection of two-fold differences in DEL frequency, and dose-dependent effects of carcinogens on deletion frequency are detected. Protocols for running the screen using readily available

robotics and instrumentation have been developed and pilot automated screens with small numbers of positive and negative controls have shown promising results. We are validating the automated assay with a set of coded chemicals of known carcinogenic activity. Automated, higher throughput and highly predictive genotoxicity screens like the DEL assay will assist in the identification of undesirable compound characteristics earlier in the drug discovery process, improving pharmaceutical companies' ability to make fast, scientifically-based decisions on early drug leads. More widespread adoption of validated yeast or mammalian cell DEL assays may reduce the number of animals used in carcinogenicity testing of novel pharmaceuticals, and its high predictive value may reduce the failure rate of promising drug candidates due to unanticipated carcinogenic potential.

363 CONSTRUCTION OF CHINESE HAMSTER OVARY CELL LINES EXPRESSING SINGLE COPY OF HUMAN N-ACETYLTRANSFERASE 2.

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Acetylation is important in the bioactivation and deactivation of aromatic and heterocyclic amine carcinogens in the diet, cigarette smoke, and the environment. These acetylation reactions are catalyzed by N-acetyltransferase-1 (NAT1) and N-acetyltransferase-2 (NAT2) that exhibit a genetic polymorphism in humans. We constructed a Chinese hamster ovary (CHO) cell system possessing human NAT2 to investigate the role of this enzyme polymorphism on aromatic and heterocyclic amine genotoxicity. Since the level of gene expression in the stable mammalian cell lines may vary due to the unpredictable integrity and chromosomal integration site of transferred cDNA and/or copy number of the cDNA, it is critical to control for these variables. Since the Flp-In expression system (Invitrogen) allows integration and expression at a specific genomic location, we selected it to express NAT2 into CHO cells. pcDNA5/FRT containing human *NAT2*4* was co-transfected with pOG44 plasmid into Flp-In-CHO cells containing an integrated Flp recombination target (FRT) site. Cells were selected for Hygromycin B resistance. Integration of the pcDNA5/FRT construct into the FRT site was tested by polymerase chain reaction (PCR) using forward and reverse primers specific for *NAT2*4* and *lacz* gene, respectively. The possibility of pcDNA5/FRT construct integration into a second genomic site was excluded by the observation of the absence of PCR product using forward and reverse primers specific for *NAT2*4* and *Hygromycin* gene. Cells that were Hygromycin-resistant with *NAT2*4* integrated into the FRT site exhibited measurable sulfamethazine NAT2 activity whereas untransfected Flp-In CHO cells did not. Integration and expression of a single copy of *NAT2*4* in CHO cells makes this Flp-In system useful for functional comparison of genetic variants of human NAT1 and NAT2 alleles. Partially supported by USPHS grant CA-34627 from the National Cancer Institute.

364 FLOW CYTOMETRIC ANALYSIS OF MICRONUCLEUS FORMATION IN RAT PERIPHERAL BLOOD: INTEGRATION WITH SUBCHRONIC DOSING STUDIES TO REDUCE ANIMAL USAGE.

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Incorporating genotoxicity endpoint(s) into subchronic toxicology studies can potentially reduce animal usage. Recent data suggests that rat peripheral blood may be a sensitive compartment for highlighting chemical-induced cytogenetic damage expressed as micronuclei (MN). Many of these experiments have utilized conventional microscopy-based inspection to score MN events. An experiment was conducted to evaluate whether a method for scoring MN by high-speed flow cytometry (FCM) provides information of equal utility as that achieved by microscopy. This FCM method utilizes an immunochemical reagent (anti-CD71-FITC), RNase, and propidium iodide to differentially stain four cell populations: normochromatic erythrocytes, normochromatic erythrocytes with MN, reticulocytes and reticulocytes with MN (commercially available as MicroFlow®PLUS). For this experiment, rats were treated with vehicle (saline) or a range of methyl methanesulfonate (MMS) concentrations 5 days per week for 4 weeks. Blood samples were drawn initially on Day 0 and then on Days 2, 3, 4, 11, 18 and 25. MN frequencies were determined by analyzing 20, 000 young reticulocytes per blood sample. Instrumentation and calibration procedures utilizing rat bone marrow and a biological model were employed, and are described. Vehicle treatment showed no change in MN-RET frequency over the experimental timeframe, irrespective of scoring methodology. For MMS treated animals, the MN-RET frequency increased in a time and dose-dependent manner. While the average MN-RET values were similar for both scoring procedures, less variation was evident with the FCM-based measurements. Note that the FCM-based data acquisition was achieved in a fraction of the time (1/10) of the microscopy-based measurements.

365 FLOW CYTOMETRIC SYSTEM FOR SCORING MICRONUCLEATED RETICULOCYTES IN HUMAN BLOOD SAMPLES.

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Micronucleated reticulocytes (MN-RET) in the peripheral blood circulation of humans may represent a valuable endpoint of cytogenetic damage. However, the human spleen is capable of sequestering and removing micronucleus-containing erythrocytes from peripheral blood circulation. Consequently, the rarity of MN-RET has made it difficult to score these events, or to effectively evaluate the sensitivity of the endpoint. Here, we describe a single-laser flow cytometric system for enumerating the incidence of micronuclei in the youngest subset of reticulocytes. The procedure is based on an immunochemical reagent specific for the transferrin receptor (CD71). When fixed cells are treated with phycoerythrin-conjugated anti-CD71, RNase, and SYTOX Green dye, the fluorescent resolution of four erythrocyte populations is achieved (i.e., young reticulocytes and mature erythrocytes, with and without micronuclei). In addition to these minimal staining requirements, the system benefits from one additional immunochemical reagent, anti-glycophorin A-CyChrome. Anti-glycophorin A labeling of erythroid cells helps ensure that debris does not adversely impact the analysis. Instrument calibration procedures utilizing malaria-infected rodent erythrocytes have also been developed, and are described here. With this system, blood samples from ten healthy non-splenectomized human volunteers were analyzed for micronucleus frequencies with a single-laser flow cytometer. Additionally, blood from three healthy splenectomized volunteers was also evaluated. The resulting data suggest that micronuclei can be quantified in the most immature fraction of human reticulocytes with a single-laser flow cytometer. This high throughput system is potentially important for evaluating the value of the MN-RET endpoint in human populations.

366 ENZYMATIC ACTIVITIES OF WERNER SYNDROME PROTEIN ON A TELOMERE TYPE SUBSTRATE.

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Werner Syndrome (WS) is a hereditary disorder that is characterized by early onset of aging symptoms and increased frequency of age-related diseases including cancer and atherosclerosis. WS is caused by mutations in a gene that codes for the Werner protein (WRN) that contains both DNA unwinding (helicase) and exonuclease activities suggesting a role in DNA metabolism. WRN belongs to the RecQ family of helicases that are involved in maintaining genomic stability. Consequently, defects in WRN lead to increased genomic instability characterized by chromosomal deletions, insertions, translocations and the accelerated loss of telomeres, the protective repeating sequences at the ends of chromosomes. In this context, WS serves as a good model for examining the relationship between accumulation of genetic changes induced by endogeneous and environmental agents during lifetime and the degenerative processes that occur during normal aging. In most normal somatic cells, telomeres shorten with each round of cell division, eventually leading to cessation of cell division known as cellular senescence. In WS cells, telomeres shorten at a much faster rate, leading to premature cellular senescence and suggesting a possible role for WRN in telomere length maintenance. Recent studies indicate that each chromosome forms a specialized structure known as T loop in which the 3'single-stranded telomeric end is inserted back into the double-stranded telomeric region. We have constructed model DNA substrates that mimic the T loop structure of telomeres and have carried out experiments to study the activities of WRN on these substrates. DNase I footprinting indicates that WRN binds specifically to the T loop structure. This binding appears to mediate disruption of the T loop structure by WRN helicase activity. In addition, WRN has a very efficient exonuclease activity on the 3' end of our T loop substrate. Our findings suggest a potential role for WRN in processing T loop structures during telomere metabolism.

367 GENOMIC INSTABILITY OF PUN GENE IN MOUSE ENBRYONIC CELLS WITH N-ETHYL-N-NITROSOUREA.

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Genomic instabilty (GI) by various chemical substances are regarded to play important roles in toxicology such as carcinogenesis, teratogenesis and germ cell mutagenesis. Therefore, detection of GI *in vivo* mammals is very important to evaluate the toxicity of chemical substances to human health. We recently adapted Pun mice to detect GI of the pun gene in embryonic somatic and germ cells. The embryonic mouse cell systems observed in this study were melanocytes (MLC) for somatic cells and primordial germ cells (PGC) for germ cells. The experimental systems used were mouse spot test and mouse specific locus test on P gene. We observed recessive mutations (RM) of P gene and intragenic recombinations (IGR) of pun gene. The

pun gene is composed of duplicated exons of P gene and it is easily reverted to wild type P gene through IGR. Homozygous pun males and heterozygous pun/P female were mated and transplacentally treated to N-ethyl-N-nitrosourea (ENU) at day 10.5 of development. The spontaneous frequencies of RM on P gene and IGR on pun gene in embryonic somatic cells are relatively high but they are very low in germ cells. ENU induced IGR and RM with high frequency in embryonic somatic cells and the frequency of IGR is much higher than that of RM. The male mice (Fo) obtained were mated with non-treated homozygous pun females and obtained the next generation (F1) to detect IGR in PGC with ENU. In F1 mice, IGR in PGC are observed with extraordinary high frequency with dose-dependency and mosaic mice were obtained. But the frequencies of IGR in MLC of F1 mice were decreased to the control level. These results demonstrate that delayed mutagenesis is occurred in the embryonic germ cell line of the mouse pun genes with ENU.

368 THE EFFECT OF DIFFERENT DNA DAMAGING AGENTS ON P53 AND MDM2 PROTEINS IN CANCER CELL LINES AND MOUSE SKIN.

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The product of p53 gene is a transcription factor for several known cell cycle regulation-linked genes. Wild type p53 protein is increased, probably by stabilization, in response to various cellular stress signals, e.g. chemically induced DNA damage. The increase of p53 is thought to be a consequence of disrupted binding of p53 to Mdm2, which is mainly responsible for the degradation of p53. The aim of this study was to find out whether Mdm2 is the cause of p53 increase after treatment with different DNA damaging agents. We used MCF-7 breast adenocarcinoma and A-549 lung carcinoma cells and an *in vivo* C57BL/6 mouse skin model to study the effects of benzo(a)pyrene, cisplatin, docetaxel and azidothymidine (AZT) on p53 and Mdm2 proteins. The amount of p53 and Mdm2 proteins were studied by Western blotting using antibodies DO7 and CM5 for p53 (human and mouse, respectively) and H-221 for Mdm2 (both human and mouse). p53 was induced by benzo(a)pyrene in both cell lines and in mouse skin. Cisplatin also induced p53 in both cell lines, while docetaxel was studied only in MCF-7 cells, and was found to induce p53. Mdm2 was generally increased along with p53. AZT, however, had no clear effect on p53 or Mdm2 in either cell lines. In conclusion, because p53 was induced by three different DNA damaging agents in these models, but not with AZT, AZT probably did not cause DNA damage, although evidence of the carcinogenicity of AZT have been presented. Increase of Mdm2 indicates that the induced p53 is in functional form and that changes of Mdm2 are not the reason for p53 induction.

369 TRP-P-1 AND TRP-P-2 MODULATE THE ACTIVITY OF POLY(ADP-RIBOSE) SYNTHETASE *IN VITRO.*

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Heterocyclic amines (HAs) are produced by pyrolysis of amino acids or proteins in fried, broiled, or grilled meats. These compounds are potent mutagens that increase rates of colon, mammary, and prostate tumours in rodent bioassays. The carcinogenic effect of these compounds is elicited from metabolic activation by the cytochrome P450 enzyme system to reactive metabolite(s). Interestingly, the P450 catalytic cycle has the absolute requirement *in vivo* of NAD(P)H. Here we report the effects of six groups of HAs on the activity of poly(ADP-ribose) synthetase (PARS), an enzyme implicated in several important biological processes, such as DNA repair, apoptosis, or necrosis. PARS activation results in an immediate decrease in available NAD+ and may limit the activity of enzymes requiring this cofactor. Our results show that 3-amino-1, 4-dimethyl-5H-pyrido[4, 3-b]indole (Trp-P-1) causes a 91% and 94% decrease at 1 and 5 mM, respectively (IC50 = 0.22 mM). In contrast, 3-amino-1-methyl-5H-pyrido[4, 3-b]indole (Trp-P-2) at 1 mM causes a 134% increase in PARS activity *versus* a 93% decrease in the activity at 5 mM (IC50 = 2.2 mM). In all other compounds tested, only an inhibitory effect was observed. The biological significance of these findings remains to be studied; however, it is possible that by inhibiting PARS, the available pool of NADH/NAD+ allows for increased activation of these compounds to toxic metabolites. (Supported by the Japan Society for the Promotion of Science)

370 THE EFFECT OF ETHANOL, METHANOL, AND DIMETHYL SULFOXIDE ON POLY(ADP-RIBOSE) SYNTHETASE ACTIVITY.

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The inherent toxicity of a compound can be masked by the choice of solvent used to deliver the chemical of interest. The purpose of this study was to determine the effect of three organic solvents [ethanol, methanol, or dimethyl sulfoxide (DMSO) and water mixtures] at modulating the activity of poly(ADP-ribose) synthetase (PARS), a key enzyme in the repair of damaged DNA. A dose-response was performed with each of the respective solvents. A 112% and 75% increase in activity above baseline was observed with 15% ethanol and 10% methanol, respectively. However, a near 20% decrease in activity was observed with 4% DMSO. Our results show an activating effect on PARS activity when ethanol or methanol is used at low concentrations, compared to the inhibitory effect of DMSO. PARS activity can deplete intracellular NAD+ and subsequently intracellular ATP, thereby causing necrotic cell death, if the capacity to replenish ATP is overcome. In constrast, apoptosis is associated with PARS cleavage and inhibition. Our data indicates that the interpretation of studies obtained when using common organic solvents may be dramatically skewed, either exaggerating the inherent toxicity of the compound or masking its potential for damage. (Supported by the Japan Society for the Promotion of Science)

371 MECHANISM INVOLVED IN TOPOISOMERASE IIα INHIBITION BY PHOTOACTIVATED BAY Y3118.

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Topoisomerase inhibition has been proposed as a possible mechanism involved in the photochemical toxicity and mutagenicity of fluoroquinolone antibiotics (FQs). We report here that some photochemically mutagenic FQs, but not all, inhibit the human topoisomerase IIα enzyme *in vitro* following UVA (365nm) irradiation. We studied Bay y3118 (y3118) and lomefloxacin, which are highly photoactive, ciprofloxacin which is weakly photoactive and lomefloxacin, which is photostable. The degree of inhibition at 360 mJ/cm2 was as follows: Bay y3118, 15%; ciprofloxacin, 5%; lomefloxacin, 0% and moxifloxacin, 0%. The nature of the UV-FQ-mediated inhibitory effects on the topoisomerase IIα was further examined using the photoinhibitory y3118. Topoisomerase IIα inhibition required the simultaneous presence y3118; DNA and enzyme in the UVA irradiated reaction mixture indicating the possible formation of a ternary structure. UVA-FQ-mediated topoisomerase IIα inhibition also correlated with the increased binding of y3118 to DNA:topoisomerase complexes and was not prevented by the antioxidant TEMPO suggesting that reactive oxygen species were not involved in the inhibition. We conclude that some photochemically genotoxic FQs inhibit the human topoisomerase IIα (i.e. Bay y3118) possibly by UV-induced affinity of FQs to DNA:topoisomerase complexes, but that inhibition of topoisomerase IIα does not account for the photochemical activity of others FQs (i.e. lomefloxacin). Supported by the NIH Grant CA 86056.

372 RESISTANCE TO SILICA-INDUCED LUNG FIBROSIS IN SENESCENT RATS: ROLE OF ALVEOLAR MACROPHAGES AND TUMOR NECROSIS FACTOR-ALPHA (TNF).

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PURPOSE: We have previously demonstrated in rat alveolar macrophages (AM), that aging was associated with a progressive decline in the ability of these cells to produce TNF 1. The purpose of the present study was to investigate the effect of aging on silica-induced lung toxicity, for which it has been demonstrated the requirement of TNF for the development of silica-induced pulmonary fibrosis2. SUMMARIZED DESCRIPTION: Sprague-Dawley rats 3 and 18 month old were intratracheally instilled with 30 mg of silica or saline as control. Two weeks following instillation, rats were analysed for bronchoalveolar lavage fluid (BAL) parameters (total protein, LDH, TNF and cellularity), total lung hydroxyproline, as measurement of collagen deposition, and lung histopathology. RESULTS: In young animals, as expected, silica induced a significant increase in BAL protein, TNF, LDH as well as in cell numbers, which correlate with increased collagen deposition and silicotic nodules formations. In old rats, however, no changes in BAL or lung parameters were observed following silica instillation. These *in vivo* results were also confirmed *in vitro*, where silica failed to induce TNF release in AM obtained from old animals. This defective response to silica could be explained with the defective

protein kinase C translocation, due to a reduction in its anchoring protein RACK-1 in old rats. Use of RACK-1 antisense oligonucleotide reduced the response of young macrophages to silica, supporting the idea that age-associated alterations in AM signal transduction pathways contribute to decreased sensitivity to silica-induced lung fibrosis.

373 THIOL ANTIOXIDANTS INHIBIT THE ADJUVANT EFFECTS OF AEROSOLIZED DIESEL EXHAUST PARTICLES IN A MURINE MODEL FOR OVALBUMIN SENSITIZATION.

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Although several epidemiological studies indicate a correlation between exposure to ambient particulate matter (PM) and adverse health effects in humans, there is still a fundamental lack of understanding of the mechanisms involved. We set out to test the hypothesis that reactive oxygen species are involved in the adjuvant effects of diesel exhaust particles (Department) in a murine ovalbumin (OVA) sensitization model. First, we tested six different antioxidants, N-acetylcysteine (NAC), bucillamine (BUC), silibinin, luteolin, trolox (vitamin E) and ascorbic acid, for their ability to interfere in Department-mediated oxidative stress *in vitro*. Of the six agents tested only the thiol antioxidants, BUC and NAC, were effective at preventing a decrease in intracellular GSH/GSSG ratios, protecting cells from protein and lipid oxidation, and interfering with HO-1 expression. We therefore selected the thiol antioxidants for testing in the murine OVA inhalation exposure model. Our data demonstrate that NAC and BUC effectively inhibited the adjuvant effects of Department in the induction of OVA-specific IgE and IgG1 production. Furthermore, NAC and BUC prevented the generation of lipid peroxidation and protein oxidation in the lungs of OVA + Department exposed animals. These findings indicate that NAC and BUC are capable of preventing the adjuvant effects of inhaled Department, and suggest that oxidative stress is a key mechanistic component in the adjuvant effect of Department. Antioxidant treatment strategies may therefore serve to alleviate allergic inflammation and may provide a rational basis for treating the contribution of PM to asthmatic disease. (Supported by US Public Health Service Grant AI34567, EPA Southern California Particle Center, and by NIAID AI07126).

374 THIOL AND IMMUNE EXPOSURE DEPENDENT RESPONSE TO OVALBUMIN IN SENSITIZED RATS.

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Brown Norway rats were exposed 30 min to the antigen, ovalbumin (OVA) at 7.2, 68, 93, 111 or 156 mg/m3 on days 1, 8, 15, and 29. Tissue was collected one day after the last exposure. Cysteine (CYSH), glutathione (GSH), and markers of inflammation in bronchioalveolar lavage fluid (BALF) were measured. Alveolar macrophage (AM) and pulmonary associated lymph node lymphocyte (PALNL) were isolated and assessed for CYSH and GSH concentrations. Specific IgG and IgE, and lung tissue IFN-gamma, IL-4, and TNF-alpha mRNA levels (standardized with G3PDH) were the immunological endpoints studied. Rats were immunologically sensitized as evident by exposure related increases in all immunological parameters and OVA challenge induced increases in BALF albumin, protein and lactate dehydrogenase. In addition, OVA caused a modest elevation at low OVA challenges, but sharp reduction in AM thiols at higher OVA challenges. Concomitantly, OVA exposure dependent increases in BALF CYSH and GSH were observed. CYSH, but not GSH was elevated in PALNL of OVA challenged rats. In summary, antigen exposure dose dependent alteration of thiol and immune parameters were demonstrated *in ova* sensitized and challenged rats.

375 PROTEASE ENHANCEMENT OF IGG1 AND IGE ANTIBODY RESPONSE TO HEMICELLULASE IN MOUSE MODELS OF ALLERGY.

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The mouse intranasal (MINT) and mouse aspiration (MaspT) tests are being developed as replacements to the guinea pig intratracheal (GPIT) test used to assess the allergenic potential of protein allergens. Protease enzymes have been shown to enhance the IgG1 antibody response to non-protease enzymes in guinea pigs exposed to mixtures of protease and non-protease enzymes (Sarlo, et.al. JACI, 1997, 100:480). The shift in the antibody response to enzyme mixtures in the GPIT test is used to adjust occupational exposure guidelines. To replace the GPIT test, we must show similar shifts in antibody responses to enzyme mixtures in the mouse models. BDF1 mice were exposed *via* intranasal (IN) instillation to protease, hemicellulase or a mixture of protease + hemicellulase on days 1, 3, 10, 17 and 24; sera were collected on day 29. Similarly, mice were exposed to the same enzymes *via* aspiration (ASP) instillation on days 1, 3 and 10; sera were collected on day 15. IgG1 and IgE antibody to each enzyme were measured by ELISA. IgE titers were randomly confirmed by the rat passive cutaneous anaphylaxis (PCA) test. The protease shifted the IgG1 antibody response to the hemicellulase ~3x in IN and ASP dosed mice. The shift was dependent upon the amount of protease in the mixture - the lower the protease levels in the mixture, the smaller the shift. The protease also shifted the IgE antibody response to the hemicellulase in IN and ASP dosed mice. The enhancement was greater for IgE than for IgG1. The antibody response to protease was not altered by the presence of the hemicellulase. Testing of the protease + hemicellulase mixture in the GPIT test also showed a 3x to 4x shift in IgG1 antibody to the hemicellulase. Therefore, there is consistency between the guinea pig and mouse models. Continued work with mixtures of enzyme allergens is needed to develop the mouse model as a full replacement to the GPIT test.

376 RESPIRATORY PHYSIOLOGICAL RESPONSES TO AN EXTRACT OF *Stachybotrys chartarum* IN BALB/C MICE.

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Exposure to *Stachybotrys chartarum* has been associated with the development of serious health problems in humans, including asthma. Previous studies by our group demonstrated respiratory exposure to a pool of *S. chartarum* extracts caused biochemical and immunological responses indicative of a respiratory allergic response. The goal of this study was to assess the ability of this extract to cause alterations in respiratory physiological responses similar to those observed in human allergic asthma. Five isolates of *S. chartarum* obtained from wallboards in water-damaged houses were grown and combined in approximately equal weight amounts, extracted using Hanks' Balanced Salt Solution (HBSS) + Tween-80, and filter sterilized to form a crude antigen preparation (SCE-1). Female BALB/C mice were anesthetized and exposed to 4 aspirations of 50 µl volume containing 10 mg of SCE-1, BSA as a non-allergenic protein, or HBSS over a 4-week period. Barometric whole-body plethysmography was performed to measure enhanced pause (PenH) 10 minutes prior to (baseline) and 1 hour following each aspiration exposure to assess immediate respiratory responses. Additionally, airway hyperresponsiveness to nebulized methacholine (MCh) was assessed on days 1 and 3 following the 4th aspiration exposure. Exposure to HBSS or BSA did not alter baseline PenH values, PenH following the aspiration exposures, or airway responsiveness to MCh. Exposure to SCE-1 resulted in a 4.7-fold increase in PenH over baseline after the 3rd exposure, increasing to 5.6-fold after the final exposure, and increased responsiveness to a 32 mg/ml MCh aerosol challenge. We conclude respiratory SCE-1 exposure causes respiratory physiological responses similar to those observed in human allergic asthma. However, BSA does not generate the respiratory physiological responses expected of an allergenic protein when administered by aspiration. (Supported by NCSU/EPA Cooperative Training Agreement CT826512010.) (This abstract does not reflect EPA policy.)

377 PATHOLOGIC AND CYTOKINE RESPONSES IN THE RESPIRATORY TRACT OF A/J MICE AFTER INTRANASAL SENSITIZATION AND CHALLENGE TO TOLUENE DIISOCYANATE.

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Occupational asthma is the most frequently diagnosed respiratory disease in the workplace and is often linked to low molecular weight chemicals (LMWC) such as toluene diisocyanate (TDI) that sensitize the respiratory tract. Occupational asthma is characterized by smooth muscle hypertrophy, mucus hypersecretion, epithelial desquamation and an inflammatory influx consisting of lymphocytes, eosinophils, and neutrophils in the airways. Many experimental models have linked the Th2 phenotype to LMWC-induced occupational asthma. Most murine models of occupational asthma, however, use systemic administration (e.g., dermal) to sensitize mice. The present study was designed to test the hypothesis that intranasal sensitization and challenge to TDI will induce the immunologic (cytokine and IgE) and pathologic responses in the lung that are characteristic of LMWC-induced occupational asthma. Only mice that were intranasally sensitized and challenged to TDI exhibited an increase in lung derived IL-4 mRNA. IL-4 mRNA expression in these animals reached 8-fold the level of control 24 hours after exposure and waned to 4-fold the control levels by 96 hours. In addition, only TDI sensitized and challenged animals exhibited an increase in total serum IgE. The total serum IgE levels

in these animals were 4-fold the level of control 24 hours after exposure and persisted at that level by 96 hours after exposure. No pulmonary lesions were found in mice of any group. TDI-exposed mice did have an acute rhinitis with associated epithelial necrosis and exfoliation. This study is the first to demonstrate that intranasal administration to an LMWC is an effective method of sensitization and results in an upregulation of a critical mediator of allergic airway disease, IL-4, within the lung after challenge (Supported in part by American Chemistry Council Grant # 0051).

378 CHARACTERIZATION OF IGE INDUCING PROTEINS FROM EXTRACTS OF *METARHIZIUM ANISOPLIAE.*

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We have previously demonstrated BALB/C mice exposed to *Metarhizium anisopliae* crude antigen (MACA) develop immune and pulmonary responses typical of allergy and asthma. Soluble factors from the fungal components (mycelium, conidia, and inducible proteases and chitinases) were combined in equal protein amounts to form the crude antigen. The fungal allergens were identified by SDS-PAGE/western blot analysis using IgE containing hyper-immune mouse serum as the source of primary antibody and traditional chromogenic enzyme substrate for detection. India ink staining detected more than 50 proteins in MACA. Of these 4-5 induce an IgE response and comprise approximately 12-15% of the MACA proteins by densitometric analysis of the India ink stained blot. When the MACA component extracts were examined individually, we determined that 4 of these proteins were detectible in the mycelium extract, and 2 were detectable in the inducibles extract (1 homologous by MW to a mycelium protein identified and 1 unique protein). However, there were no IgE inducing proteins detected in the conidia extract. We have begun to re-assess these extracts using a chemiluminscence detection system that is more sensitive. The IgE-inducing proteins in the mycelium extract identified as *met a* I, *met a* II, *met a* III, and *met a* IV have apparent molecular weights of 127.6 kDa, 114.7 kDa, 89.7 kDa, and 52.6 kDa, respectively. Furthermore, we have determined the isoelectric points of the allergen proteins by isoelectric focusing (IEF) and confirmed the apparent molecular weights by two-dimensional gel analysis. Using SDS-PAGE/western blot analysis we have also found batch-to-batch variability in our fungal extracts. Although we have found there are apparent changes in the concentration of the allergenic proteins in different extract batches, there do not appear to be differences in the proteins detected. (This abstract does not reflect EPA policy.)

379 EFFECT OF ORGANIC PARTICLE EXPOSURE ON ALLERGIC AIRWAY DISEASE IN MICE.

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Past studies have documented the adjuvant effect of diesel exhaust and carbon black (CB) particles on allergic airway disease (AAD) in mice immunized to elicit a TH2 lymphocyte phenotype. However, few studies have examined the effect of particle exposure on reversing the normal hyporesponsive state in animals immunized to elicit a TH1 lymphocyte phenotype. To investigate if CB exposure will alter the hyporesponsive TH1 phenotype to a responsive TH2 phenotype, mice were exposed to CB by intranasal (IN) instillation during the challenge phase of ovalbumin (OVA) induced AAD. Mice were immunized with either OVA-ALUM (TH2 phenotype) or OVA-CFA (TH1 phenotype) and then challenged with OVA aerosol (1 hr/day) for 3 days. Mice received 200ug CB IN 4 hrs prior to each OVA aerosol challenge. Animals were killed 24 hrs later and evaluated for BAL cell number and type. Mice expressing a TH1 response to OVA demonstrated significantly fewer eosinophils than mice expressing a TH2 phenotype (50% *versus* 87%) confirming the hyporesponsive state. Contrary to expectations, administration of CB to TH1 mice decreased total BAL cell number with no change in % eosinophils (50% *versus* 51%). Administration of CB to TH2 mice also decreased total cell number and proportion of eosinophils from 87% to 48%. These data suggest that CB exposure decreases the pulmonary allergic response in both TH1 and TH2 phenotype mice. (Funded by ES NIH09433)

380 OXAZOLONE-INDUCED DIRECT PLNA REACTIONS ARE SUPPRESSED IN MICE ORALLY PRETREATED WITH OXAZOLONE.

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There is a need for preclinical assays that can identify low molecular weight compounds (LMWCs) with the potential to cause systemic hypersensitivity (SH). One such assay being examined is the rodent popliteal lymph node assay (PLNA). Based on >80 LMWCs, in general, only LMWCs associated with SH cause PLN reactions in rodents. However, despite this preliminary success, there are fundamental questions that remain unanswered. One such question is concerned with false positives. For example, it is hypothesized that contact sensitizers (CS) will produce strong PLN reactions but are not expected to cause SH following oral administration due to oral tolerance. The objective of this study was to examine the immunomodulating potential of the CS oxazolone (OX) and DNCB in the BALB/c PLNA, and to examine what effect oral pretreatment with OX had on the reactions. In naïve mice, both CSs induced strong PLN reactions. OX induced dose-dependent increases in PLN weight, cellularity, the % of CD4+ T cells, and the % of B cells, with a concomitant decrease in the % of CD8+ T cells. Next, the PLNA was conducted in mice gavaged 3 times with OX. Mice gavaged with OX had suppressed PLN reactions following the injection of OX (decreased PLN size, weight and cellularity), as well as a decrease in the % of CD4+ helper T cells. Oral treatment with OX had no observable effect on the PLN reaction induced by DNCB. Finally, adoptive transfer studies were conducted to examine the mechanism of PLN unresponsiveness. It was found that unfractionated splenocytes isolated from mice gavaged with OX adoptively transferred (iv) PLN unresponsiveness to naïve mice. Collectively, these observations demonstrate that OX is a potent immunostimulator in the PLNA. As importantly, these results demonstrate that the immunostimulating potential of OX is significantly decreased in mice orally exposed to the drug, possibly due to the presence of a cell-mediated mechanism of oral tolerance.

381 CHARACTERIZATION OF THE SENSITIZING POTENTIAL OF A STRONG DERMAL IRRITANT USING AN ENHANCED LOCAL LYMPH NODE ASSAY.

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Strong irritants can give ambiguous results in animal models used for predicting dermal sensitization potential. An enhanced LLNA protocol was devised to better characterize the sensitization potential of a long chain alkylamine (LCAA), a strong dermal irritant that had previously given conflicting results in other sensitization assays. In this study, groups of five CBA mice were treated by topical application of LCAA (0.1, 0.3, 1.0% v/v) once daily to the dorsum of each ear for three consecutive days. Additional groups were treated with the known sensitizers DNCB (0.25% w/v) or HCA (50% v/v), or the non-sensitizing irritant SLS (25% w/v). Five days after the initial dose, the mice were injected (i.p.) with 5-bromo-2'-deoxyuridine (BrdU) to label proliferating cells. Auricular lymph nodes were isolated and the number of BrdU+ cells was determined for individual animals by flow cytometry. Immunophenotype analysis of the nodal cells was conducted using the marker combinations B220/CD3 and IA/CD69 to determine the B:T cell ratio and the activation state of the nodal lymphocytes respectively. Ear thickness was also evaluated for all animals. LCAA caused a significant increase in ear thickness and a dose-dependent increase in lymph node cell proliferation with a maximum Stimulation Index (SI) of 125.9 (EC3 < 0.1%), while the known sensitizers DNCB and HCA gave a SI of 104.6 and 30.1 respectively. Both positive control substances and higher dose levels of LCAA also caused a significant increase in the B:T cell ratio and the % IA+/CD69+ cells. Treatment with SLS resulted in significant ear swelling and an SI of 3.2 but no increase in cellular markers. Despite the significant irritant response induced by LCAA, the magnitude of the proliferative response and the activation state of cells localized in the nodes of treated mice identify LCAA as a potential dermal sensitizer.

382 RELEVANCE OF HUMAN CYP450 1A1 AND 1B1 IN THE METABOLISM OF FRAGRANCES.

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Fragrances are widely encountered in our daily environment and are known to be a common cause of allergic contact dermatitis. Such small molecular compounds usually need binding to high molecular weight compounds in order to become antigenic. The knowledge about the metabolic activation is still limited. Recently, we found that the proliferative responses of lymphocytes of fragrance-allergic patients were increased in the presence of human liver microsomes or recombinant CYP1A1 (Sieben et al., 2001). In addition, we found that eugenol but not isoeugenol produced a 20-fold increase in CYP1A1 steady state mRNA levels in human primary keratinocytes (Al Masaoudi et al., 2001). With the aim of providing further insights into the mode of action of eugenol (4-allyl-2-methoxyphenol) and the structurally related compound isoeugenol (4-propenyl-2-methoxy-phenol) in human skin cells, we studied whether CYP1B1, AhR and ARNT are also involved in the activation of these compounds. We found, that eugenol additionally produced a 4-fold increase in CYP1B1 steady state mRNA levels but no induction

of the AhR gene battery. In contrast, incubation of the cells with isoeugenol increased CYP1B1 and AhR mRNA levels in keratinocytes. Our results indicate that keratinocytes can activate small haptens such as fragrances. This work was supported by the BMBF (IZKF BIOMAT, G11) and the European Community.

383 ELUCIDATING CHANGES IN SURFACE MARKER EXPRESSION OF DENDRITIC CELLS FOLLOWING ALLERGEN TREATMENT.

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Dendritic cells (DC) are specialized antigen-presenting cells (APC) located in many non-lymphoid tissues, and Langerhans cells (LC), a specialized form of DC, are found in the skin. LC play a critical role in the induction of contact dermatitis and therefore have become a focal point for development of *in vitro* cell-based methods for contact sensitization testing. Because of the low abundance of skin-derived LC, cultured DC from peripheral blood (PB) are used as LC surrogates to study the effects of sensitizing chemicals on APC. It has been reported that chemical allergens induce changes in the expression of various DC surface markers. For the work presented here, DC were derived from the KG-1 cell line (KG-1 DC) or were differentiated from human PB (PBMC-DC). The phenotype of these DC was measured by flow cytometry after 48 hr treatment with the chemical allergens dinitrofluorobenzene (DNFB) and methylchloroisothiazolinone/methylisothiazolinone (MCI/MI), the irritant sodium dodecyl sulfate (SDS), lipopolysaccharide (LPS), and tumor necrosis factor-α (TNFα). It was found that only MCI/MI upregulated CD86 expression in KG-1 DC, whereas TNFα upregulated CD54 and slightly upregulated CD80 and CD86 expression. In PBMC-DC, MCI/MI and DNFB induced a slight upregulation of class II major histocompatibility expression (HLA-DR), whereas LPS and TNFα significantly upregulated CD54 and slightly upregulated CD80 and HLA-DR expression. SDS had no effect on surface marker expression in either KG-1 DC or PBMC-DC. Changes in surface marker expression in PBMC-DC treated with chemical allergens were detected in 2 of 5 donors, suggesting a limited sensitivity of PBMC-DC under these culture conditions. Futhermore, the presence of cytokines during allergen treatment masked the ability to detect changes in surface marker expression. Our data suggest that, under these culture and treatment conditions, measurement of surface marker changes using PBMC-DC or KG-1 DC does not provide a sensitive method with sufficient dynamic range for assessing the contact sensitization potential of a chemical.

384 DIVERGENT IMMUNOLOGICAL RESPONSES FOLLOWING GLUTARALDEHYDE EXPOSURE.

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Glutaraldehyde (Glut) is a potent contact sensitizer, additionally numerous cases of occupational asthma related to Glut exposure have been reported in recent years. The purpose of this study was to examine the Dose-Response relationship between Glut exposure and the development of Th1 *vs.* Th2 responses using a murine model. Initial evaluation of the sensitization potential was conducted using the standard Local Lymph Node Assay in CBA mice. Female BALB/c mice (N=5-8 per group) were used for all other studies. The IgE inducing potential was evaluated by phenotypic analysis of IgE+B220+ cells in lymph nodes draining the site of topical exposure and measurement of total serum IgE levels. The Mouse Ear Swelling Test (MEST) was used to evaluate the potential of Glut to elicit urticaria (30 min post challenge) and contact hypersensitivity (24 and 48 hrs post challenge). A dose dependent increase in lymphocyte proliferation as measured by 3H-thymidine incorporation and the %B220+ cells was observed following exposure to 0.1, 0.75, and 2.5% Glut with a positive response (SI>3 in LLNA) being observed at all concentrations tested. The MEST demonstrated an immediate response (Th2) in animals induced and challenged with 2.5% Glut, whereas animals induced with 0.1 or 0.75% and challenged with 2.5% exhibited a delayed response (Th1) at 48 hrs. post challenge. In agreement with the MEST results, only the 2.5% dose group had a significant increase ($p<0.01$) in serum IgE levels and % IgE+B220+ draining lymph nodes cells. The results of these studies indicate that the induction of a Th1 *vs.* Th2 response following dermal exposure to Glut is at least in part mediated by the exposure concentration. These studies were supported in part by NIEHS intra-agency agreement #Y1-ES-0001-01.

385 RANKING OF ALLERGENIC POTENCY OF LATEX CHEMICALS IN A MODIFIED LOCAL LYMPH NODE ASSAY.

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A modified local lymph node assay (LLNA) with *ex vivo* tritium thymidine (3H-TdR) labelling of the proliferating lymph node cells was used for determination of the allergenic potency of chemicals used in the production of latex medical gloves. Fifteen chemicals, known to induce allergic contact dermatitis in man, including various thiuram, carbamate, and benzothiazole compounds and one amine were tested. The EC3 (effective concentration inducing a threefold increase in proliferation of lymph node cells (Stimulation Index, SI=3)) was calculated with non-linear regression analysis, including a bootstrap method for determination of the 5%-95% confidence interval of the EC3 value. The compounds tested were thiurams: TMTM (tetramethylthiurammonosulfide), TMTD (tetramethylthiuramdisulfide), TETD (tetraethylthiuramdisulfide), TBTD (tetrabutylthiuramdisulfide), PTD (dipentamethylenethiuramdisulfide), and PTT (dipentamethylenethiuramtetrasulfide); carbamates: ZDMC (zinc dimethyldithiocarbamate), ZDEC (zinc diethyldithiocarbamate), ZDBC (zinc dibutyldithiocarbamate), ZPC (zinc pentamethylenedithiocarbamate); benzothiazoles: MBT (2-mercaptobenzothiazole), MBTS (2, 2-dibenzothiazyldisulfide), ZMBT (zinc mercaptobenzothiazole), and MBI (mercaptobenzimidazole); and DEA (diethylamine). This procedure identified 14 out of the 15 chemicals tested as sensitiser, while for one chemical, ZDBC, no EC3 could be calculated due to low responses and a lack of a Dose-Response relationship in the data obtained. The ranking order of the chemicals with increasing EC3 values (and thus decreasing allergenic potency) was found to be: ZDEC, TMTD, TETD, ZPC, ZDMC, MBTS, PTD, TMTM, MBT, MBI, PTT, ZMBT, TBTD, DEA, and ZDBC. Our results indicate that the chemicals of choice for use in latex production would be for the thiuram compounds TBTD, for the carbamates ZDBC and for the benzothiazoles ZMBT. However, one has to be aware that besides potency also the total amount of residual chemical present in the final product is important for allergy induction.

386 STUDY TO DETERMINE THE PRESENCE OF ANTIPOLYMER ANTIBODIES IN A GROUP OF DUTCH WOMEN WITH A SILICONE BREAST IMPLANT (SBI).

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Recently the presence of antipolymer antibodies (APA) has been reported to be correlated with the disease status in silicone breast implant (SBI) recipients (and fibromyalgia patients. A cross sectional study was conducted in a population of Dutch women with SBI and self reported severe complaints. SBI recipients (n=42) were clinically examined and blood samples were obtained. In the APA assay the binding of immunoglobulins to acrylamide polymer-coated strip (2) was demonstated after incubation with secondary antibodies and visualization by a colorimetric method. In 12 of 42 SBI recipients an increase in the level of polymer binding immunoglobulins was detected compared to a negative reference sample, 3 of these 12 showing a positive and 9 a weakly positive response. In 3 out of 12 non-SBI recipients, included for control on the performance of the APA assay, an increased level of polymer binding immunoglobulins was demonstrated, 2 of these 3 showing a positive and 1 a weakly positive response. In the general population in 5 out of 80 women a positive APA was found, and one of 80 showed a weakly positive response. The study population of SBI recipients was categorised in severity subgroups (limited, mild, moderate, advanced) based on the functional capacity and the physicians general assessment of pain and disease activity. Most (34 of 42) SBI recipients belonged to the limited severity subgroup. In conclusion, we did not find a high prevalence of polymer binding immunoglobulins (APA) in a group of Dutch SBI recipients with self reported severe complaints. A discrepancy was present between the self-reported severe complaints and the observed mild clinical symptoms. SBI exposure (mean 17 years) did not result in induction of polymer binding immunoglobulins in this minimal symptomatic study group. Antigen strips for the APA assay were kindly provided by Dr R.B. Wilson, Autoimmune Technologies, L.C.C., New Orleans, LA, USA.

387 EXPOSURE TO SINGLE OR TERTIARY MIXTURES OF N, N-DIETHYL-M-TOLUAMIDE (DEET), PYRIDOSTIGMINE BROMIDE (PYR), JP-8 JET FUEL AND STRESS IN AUTOIMMUNE PRONE MICE.

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Exposures to chemicals such as DEET, PYR, and JP-8 jet fuel (JP-8) are common in military environments, as well as in some occupations. During the Gulf War, military service personnel were exposed to PYR, DEET, JP-8, and stress. Previous studies determined the lowest adverse effect level of immunological parameters to be 15.5 mg/kg DEET, 2 mg/kg PYR, 500 mg/kg JP-8, and 20 minutes exercise stress for 14 days. To investigate the contribution of these agents in accelerating or exasperating autoimmune disease, a strain of autoimmune prone mice, MRL lpr/lpr, were exposed for 14 days at age 12 weeks. At 18 weeks of age, immunological parameters in mice were assessed. No remarkable changes occurred in complete

peripheral blood counts, spleen and thymus total cellularity and CD4/8 flow cytometry, except for a decrease in thymic CD4-/CD8+ cells after exposure to JP-8 only. Furthermore, macrophage nitrite production or splenic lymphocyte proliferation after exposure to concanavalin A or lipopolysaccharide was not significantly different. Proteinuria was also assessed in these mice at 14, 16 and 18 weeks. By 18 weeks of age, MRL-lpr/lpr female mice had an expected time-dependent increase in proteinuria. Following single exposure to either PYR or exercise stress, an increase in proteinuria was noted over controls. Single exposures to DEET or JP-8 minimally affected the development of proteinuria as compared to controls. Furthermore, mice exposed to the stress mixture (20 min of exercise + 15.5 mg/kg DEET + 2 mg/kg PYR) or JP-8 mixture (500 mg/kg JP-8 + 15.5 mg/kg DEET + 2 mg/kg PYR) demonstrated increased proteinuria over control values, but this change was not typically greater than levels achieved after single exposure to PYR or stress. Thus far, these data suggest that several immunological endpoints were not remarkably exacerbated in an autoimmune mouse model, however proteinuria may be accelerated.

388 THE POSSIBLE ROLE OF THE GUT IN D-PENICILLAMINE-INDUCED AUTOIMMUNITY IN THE BROWN NORWAY (BN) RAT.

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In male Brown Norway rats, high dose D-penicillamine (D-Pen; 20mg/day) induces an autoimmune syndrome in =70% of animals within 6 to 8 weeks that is similar to drug-induced lupus in humans. The gut is believed to be central to the induction of oral tolerance. Low doses induce tolerance, while high doses induce autoimmunity. In the Brown Norway model, a tolerance-producing regimen consisting of a two-week period of low dose D-pen (5mg/day) followed by high dose D-pen results in none of the animals developing lupus. The hypothesis to be tested is that gut immunostimulation by high dose D-Pen treatment could provoke the development of autoimmunity. Male Brown Norways (n=3) were given 20 mg/day of D-Pen for periods of 0, 48 hrs and 1 week. Immunohistochemistry was performed on frozen sections of the caecum and the large intestine to examine the expression of ED1+ macrophages and B7.2, a major costimulatory molecule involved in initiating immune responses. These early time periods were chosen as changes in the distribution of ED1+ and B7.2+ cells will reflect changes in response to the drug, rather than simply reflecting signs of disease. In the caecum, both B7.2+ and ED.1+ expressing cells start to infiltrate the gut after only 48 hours of exposure to high dose D-Pen. By one week, large areas of infiltration are apparent in all sections. In the large intestine, similar findings are apparent. Interestingly, exposure to D-Pen results in the expression of B7.2 by epithelial cells. These data indicate a possible role of the gut in initiating D-Pen-induced autoimmunity.

389 PENICILLAMINE INDUCED LUPUS-LIKE SYNDROME: INVESTIGATING THE ROLE OF COVALENT BINDING *IN VIVO*.

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BACKGROUND: Idiosyncratic drug reactions are often explained by the Hapten Hypothesis, i.e. covalent binding of a drug or its metabolite to proteins leads to an immune response. We used an animal model, penicillamine-induced autoimmunity in the Brown Norway rat, to study the relationship between covalent binding and the mechanism of an idiosyncratic reaction. METHODS: Antibodies against penicillamine were induced in rabbits by injecting an penicillamine-KLH antigen. Using ELISA, the rabbit serum showed specificity for penicillamine at dilutions as high as 1/100 000. RESULTS: Male Brown Norway rats were given penicillamine (20 mg/day in water). When animals developed evidence of autoimmunity, the animals were killed and the spleen, liver and skin tissues were analyzed by immunohistochemistry and western blotting. Immunohistochemical analysis revealed low binding in spleen and liver of sick rats, but much higher binding was observed in collagen fibers of the skin. Western blot analysis under reducing conditions revealed no specific binding, but under non-reducing conditions, we identified several protein species that were covalently bound by penicillamine. This indicates that the binding involved a disulfide linkage. The molecular weight of the proteins bound by penicillamine ranged between 48 and 94 KDa. Attempts at finding autoantibodies against collagen were unsuccessful because of high background binding of the rat serum. DISCUSSION: It has been reported that penicillamine can bind aldehydes on collagen and cause fragility of bones, skin and delay wound healing. Due to the chemical structure of penicillamine, we expected that binding would occur with all sulfydryl-containing proteins and not just collagen. We suspect that penicillamine is being removed enzymatically or non-enzymatically. The high degree of binding to collagen may be because enzymes that catalyze an exchange are mostly intra-cellular. The role of binding of penicillamine to collagen in the pathogenesis of this idiosyncratic reaction is currently under investigation.

390 EVALUATION OF AN ENVIRONMENTAL CONTAMINANT, CADMIUM, ADMINISTERED IN DRINKING WATER IN THREE AUTOIMMUNE MODELS.

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The etiology of autoimmune disease is largely unknown. However, there is evidence that there are genetic factors that predispose some individuals. When these people are assaulted with environmental triggers such as drugs, viruses, or chemicals, then autoimmune disease can result. The purpose of these studies was to determine if cadmium (Cd) was a potential candidate for producing autoimmune disease (AD) in genetically predisposed individuals. NZB/W and MRL/lpr mice (exacerbation models) and Brown Norway (BN) rat (induction model for systemic lupus erythematosus) were utilized. NZB/W: Mice were exposed to Cd in the drinking water at concentrations of 0 to 10, 000 ppb for 31 wks. At 15 wks of exposure, the Cd treated groups showed onset of AD, i.e. proteinuria, compared to 27 wks for tap water controls. Anti-nuclear antibody (ANA) levels were increased after 31 wks of exposure in the 30 and 300 ppb groups. Splenocytes cultured from mice in the long-term study showed increases in IgG2a & IgG3 antibodies. In the serum, a similar trend was observed. MRL/lpr: Mice were exposed to 0 to 10, 000 ppb Cd for 11 wks. Cd-treated groups developed proteinuria 3 wks earlier than the vehicle group and a larger percentage developed ANA. Deposition of immune complexes in the glomerulus was confirmed by electron microscopy. BN: Rats were exposed to 0 to 300, 000 ppb Cd for nine wks. Increases in the number of rats positive for ANA were observed in the Cd dose groups. The level of urinary Cd was significantly increased in the high dose group; notably, the level is 500-times lower than that reported to produce renal lesions from Cd. Based on the development of ANA, and the low levels of Cd in the urine, Cd may be capable of inducing autoimmunity in the BN. The findings from these rodent models suggest low levels of Cd in drinking water have the potential to exacerbate AD. Supported in part by NIEHS contract 05454.

391 MERCURY ACCELERATES AUTOIMMUNE DISEASE IN THE GRAFT *VERSUS* HOST DISEASE (GVHD) MODEL.

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Mercury (Hg) is immunotoxic in many species, and has been shown to elicit immunologicaland physiological effects similar to lupus, an autoimmune disease, in inbred rodent strains. We have utilized GVHD, a well characterized model for lupus-like autoimmune disease, in which lymphocytes from a DBA/2 parent are injected into a C57Bl6xDBA/2-F_1 offspring host. This model, an alloantigen driven *in vivo* anti-host response, permits evaluation of Th1 and Th2 mediated responses in the development of disease. In this study, both donor and recipient mice were pretreated on alternate days, for 3 wks, by s.c. injection of $HgCl_2$ at either 20 or 200 µg/kg. This treatment does not affect body weight or survival, but has been shown to alter Th1/Th2 signalling without affecting splenocyte subsets. WIthin 5 days after the last Hg treatment, lymphocyte transfer was done and disease then assessed by monitoring prteinuria, antibody levels, and mortality. Four groups of female mice were studied: control C57BL6xDBA/2-F_1 animals; GVHD animals; GVHD+20 µg/kg Hg; and GVHD+200 µg/kg Hg. Mercury pretreatment significantly increased risks of early mortality in the GVHD model. At 4 months after disease initiation, 4 Hg animals had died (2 in the high group, followed by 2 in the low group) as compared to none in either control group. Also, Hg treatment significantly increased the likelihood of early and more severe proteinuria, as compared to the controls or the untreated GVHD group. No changes were observed after 8 weeks in serum autoantibody (anti-ssDNA IgG) levels; however further analyses will be conducted to measure specific autoantibodies. The results indicate that Hg may accelerate or worsen clinical autoimmune disease in susceptible populations. *Support from the Heinz Family Foundation and the Maryland Chapter of the Arthritis Foundation.*

392 SILICA ACCELERATED SYSTEMIC AUTOIMMUNE DISEASE IN LUPUS PRONE NEW ZEALAND MIXED MICE.

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The genetic backgrounds of lupus-prone murine models are a valuable resource for studying the influence of environmental exposure on autoimmune diseases in sensitive populations. Genetic predisposition may be a prerequisite for the develop-

ment of spontaneous autoimmune disease, such as systemic lupus erythematosus (SLE), but the incomplete concordance of SLE in twins suggests that exogenous or environmental factors are also important. Epidemiological studies have shown associations between silica exposure and several autoimmune diseases, including scleroderma and ANCA-associated small-vessel vasculitis. To determine whether long-term silica exposure exacerbates systemic autoimmunity in genetically predisposed animals, New Zealand mixed mice were intranasally instilled with saline or saline suspensions of 1 mg silica. Biomarkers of autoimmune disease were measured every two weeks by ELISA. These included circulating immune complexes, autoantibodies to nuclear antigen (ANA), histone, and double stranded DNA. ANA levels measured by ELISA were significantly higher in silica treated animals compared to saline treated animals (0.444 ± 0.17 *vs.* 0.293 ± 0.03, $p < .05$) 10 weeks post-exposure. Autoantibodies to histone were also significantly elevated after 10 weeks in silica treated animals compared to saline treated animals (0.587 ± 0.24 *vs.* 0.334 ± 0.045 $p < .05$). Autoantibodies to dsDNA were significantly elevated after 8 weeks in silica treated animals compared to saline treated animals (1.117 ± 0.305 *vs.* 0.593 ± 0.231 $p < .05$). Circulating immune complexes were seen in 6 of 8 silica treated mice, while none were seen in the saline treated mice at 10 weeks following exposure. Therefore, although NZM mice are susceptible to SLE, silica exposure significantly elevated levels of autoantibodies to nuclear antigens, including histone and dsDNA, as well as increasing circulating immune complexes, suggesting an exposure-induced exacerbation of the course of disease. This work was supported by NIH grant ES-04804.

393 ASCORBIC ACID AND GLUTATHIONE REDUCE 2-AMINO-4, 5-DICHLOROPHENOL (2A45CP) TOXICITY IN RENAL CORTICAL SLICES FROM MALE FISCHER 344 RATS.

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2A45CP is an aromatic ring hydroxylated metabolite of 3, 4-dichloroaniline. 3, 4-Dichloroaniline is nephrotoxic with primary damage located within the proximal tubules. This study assessed the *in vitro* toxicity of 2A45CP in renal cortical slices and second, determined the effect of antioxidants and sulfhydryl containing agents on the severity of 2A45CP toxicity. Renal tissue was isolated from male Fischer 344 rats (190-22 g). Renal slices were rinsed 3 times for 3 min each in 5-ml renal Krebs buffer. Tissues were incubated for 90-120 min with 0-1 mM 2A45CP. In a separate series of experiments, the slices (50-100 mg) were preincubated for 30 min with 1 mM dithiothreitol (DTT), glutathione (1 mM) or 2mM ascorbic acid prior to exposure to 0, 0.05, 0.1 or 0.25 mM 2A45CP. 2A45CP produced a concentration and time dependent increase in LDH leakage from renal cortical slices. Total glutathione levels were diminished by 0.5 mM 2A45CP within 60 min. Renal slices incubated for 60 and 120 min with 0.05 and 0.1 mM 2A45CP had lower malondialdehyde levels than control. Pretreatment with DTT did not alter 2A45CP toxicity. 2A45CP toxicity was reduced by pretreatment with GSH and ascorbic acid. These findings indicate that 2A45CP is directly toxic to renal cortical slices and that cytotoxicity is at least partially mediated by a reactive intermediate. (Supported by NIH Grant ES08834).

394 NEPHROTOXIC EFFECTS OF THE R- AND S-ENANTIOMERS OF N-(3, 5-DICHLOROPHENYL)-2-HYDROXYSUCCINIMIDE (NDHS) AND ITS SULFATE CONJUGATE.

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The agricultural fungicide N-(3, 5-dichlorophenyl)succinimide (NDPS) induces nephrotoxicity characterized by polyuric renal failure and proximal tubular necrosis. Recent findings suggest that NDPS nephrotoxicity is mediated, at least in part, through sulfate conjugates of oxidative NDPS metabolites. We have also shown that the stereochemistry of NDPS metabolites may also be an important determinant for NDPS nephrotoxicity. The purpose of this study was to determine if stereochemistry is a factor in the nephrotoxicity induced by an NDPS metabolite N-(3, 5-dichlorophenyl)-2-hydroxysuccinimide (NDHS) *in vivo* or the O-sulfate conjugate of NDHS (NSC) *in vitro*. Male Fischer 344 rats (4/group) were administered R- or S- NDHS (0.05, 0.1 or 0.2 mmol/kg) or vehicle ip. Renal function was then monitored for 48 hr. R-NDHS (0.1 or 0.2 mmol/kg) had little effect on renal function, with only minor changes in glucosuria, proteinuria and elevated blood urea nitrogen (BUN) noted at the higher dose level. In contrast, S-NDHS induced minor renal effects at 0.05 mmol/kg, but marked nephrotoxicity at 0.1 mmol/kg. The nephrotoxic potential of R- and S-NSC (0.5, 0.75 or 1.0 mM) was determined using freshly isolated renal cortical cells (3-4 million cells/ml) from untreated male Fischer 344 rats. Cytotoxicity was determined by measuring lactate dehydrogenase (LDH) release at the end of a 60-min incubation. R-and S-NSC-induced LDH release was similar at all concentrations tested, with R-NSC increasing LDH release slightly more than S-NSC at the 1.0 mM level. These results support the conclusion that stereochemistry is a factor for NDPS metabolite nephrotoxicity and that the role of stereochemistry occurs outside the kidney for nephrotoxic sulfate conjugates. Supported by NIH grant DK31210.

395 METALLOTHIONEIN ISOFORM 3 AND PROXIMAL TUBULE VECTORIAL ACTIVE TRANSPORT.

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Metallothionein isoform 3 (MT-3) has been shown to be expressed in the proximal tubule cells of the human kidney. The goal of the present study was to further characterize the basal expression of MT-3 in the proximal tubule and to determine if MT-3 participates in the maintenance of proximal tubule cell function. Expression of MT-3 mRNA was determined in the intact proximal tubule using microdissection and RT-PCR. MT-3 mRNA was detected in the proximal tubule of the *in situ* kidney with relative expression in excess to that of the beta-actin housekeeping gene. The mortal HPT cells were shown to express both MT-3 mRNA and protein and to form domes, while the immortal proximal tubule cell line HK-2, was shown to have no expression of MT-3 mRNA and protein and did not form domes. The stable transfection of MT-3 in the HK-2 cells restored MT-3 expression and dome formation in these cells. These results demonstrate that MT-3 mRNA is present in the human proximal tubule and suggest that MT-3 expression is involved in the transport function of a human renal cell line that retains properties of the proximal tubule.

396 DIFFERENT EFFECTS OF OCHRATOXIN A AND B ON ENZYME ACTIVITY *IN VITRO*.

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Ochratoxin A is a toxic secondary metabolite produced by several Aspergillus and Penicillium species. It has been reported to be primarily nephrotoxic and causally related to urinary tract tumors. Effects in the liver have also been reported. Several studies have shown the influence of OTA on hepatic glycogen levels but with contradicting results. On the one hand OTA has been demonstrated to cause hyperglycemia *in vivo*, while on the other hand, other groups have observed decreased activity of glycogenolytic enzymes *in vitro*, following OTA exposure. The glycogen content of the kidney is suggested to play an important role in the provision of energy, especially under the anaerobic conditions of the inner medulla. Thus, an influence of OTA on renal glycogen levels could be involved in its nephrotoxic action. Nothing is known about the influence of the less toxic analogue, OTB on either renal or hepatic carbohydrate metabolism. As the muscle phosphorylase is predominant in the rat kidney and also dominant in the kidneys of several other species, the influence of OTA on this isozyme was studied. OTA and OTB were covalently coupled to horseradish peroxidase (HRP) and used similarly to an antibody for dot blot analysis of glycogen phosphorylase binding. Enzyme-binding was observed for both substances. Phosphorylase itself cannot be measured directly thus, activity was measured using a combined optic test with glucose-6-phoshate-dehydrogenase (Glu-6-PDH) as indicator enzyme. Inhibition of the enzyme reaction was observed only with high (>75µM) OTA concentrations. This "inhibition" was however, actually due to inhibition of the indicator enzyme Glu-6-PDH. In contrast, Glu-6-PDH was not inhibited by OTB. The findings of this study yield no clear evidence of a specific influence of OTA on kidney carbohydrate metabolism but suggested OTA to be able to influence enzyme-activity in general. Moreover they support the strict structural requirements for OTA and OTB activity, implying either a weaker binding to, or faster dissociation of OTB from the enzyme.

397 SEX- AND AGE-SPECIFIC EFFECTS OF OCHRATOXIN A IN PRIMARY HUMAN KIDNEY CELLS (HKC).

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OTA has been demonstrated to be nephrotoxic and carcinogenic in rodents and nephrotoxic in pigs, with distinct species- and sex-differences in susceptibility. Chronic dietary intake of ochratoxin A (OTA) *via* contaminated food, leading to serum concentrations in the nM range, has also been associated with increased incidences of urothelial tumors (UT) and nephropathy in humans (e.g., Balkan Endemic Nephropathy, BEN). Epidemiological studies of the regions endemic for

BEN and UT have suggested that women are slightly more affected than men. Previous results in our laboratory have indicated OTA to disrupt the cell cycle of primary porcine renal epithelial cells. Therefore, to investigate the mechanism(s) of OTA-induced nephropathy and cancer in humans, the antiproliferative and cell cycle effects of exposure to OTA (18 and 48 hrs) were investigated in primary human male and female renal epithelial cells (HKC). To assess potential age- and sex-related differences in sensitivity to OTA, cells from both sexes and from several age groups were investigated. Average donor ages were 65.8 ± 9.6 (n=4) and 62.5 ± 13.6 (n=4) for male and female respectively. A further sample was obtained from a 14 year-old male donor. Following cell preparation from biopsy material and characterization for epithelial origin, the antiproliferative effects of OTA were determined using a standard nuclei counting procedure (crystal violet). First statistically significant proliferative arrests were found after 48 hrs at concentrations as low as 10 nM (female) and 50 nM (male) OTA. HKC obtained from the 14 year-old boy were more sensitive than those from all other male donors, showing first effects at 10 nM OTA. These results imply female HKC to be slightly more sensitive to OTA than their male counterparts and furthermore, suggest that children may be more susceptible to the toxic effects of OTA.

398 CYTOTOXICITY OF OTA AND OTB IN HUMAN AND PORCINE RENAL CELLS AFTER REPEATED EXPOSURE.

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Ochratoxins induce porcine nephropathy (PMN) and are thought to be responsible for a similar human disease, namely Balkan Endemic Nephropathy (BEN).Various mechanisms of toxic action have been proposed. Due to previous *in vitro* observations, showing that even after exposure to high concentrations of ochratoxins (≧ 10 μ M) a certain number of cells always survive, one thesis proposes that normal renal epithelial cells may be converted to fibroblasts following repeated exposure to ochratoxins. The aim of the study was to characterize the differences of these "resistant" cells compared to control cells.Thus, primary human and porcine renal epithelial cells were repeatedly exposed to acutely toxic concentrations of ochratoxin A (OTA) and its structural analogue ochratoxin B (OTB). For comparison with previous results, human (IHKE) and porcine (LLC-PK1) continuous cell lines were tested in parallel. Repeated (x3) exposures of ≧ 96 hours ensured survival of only the most resistant cells. These were used for further investigations and compared with untreated control cells.The morphology, growth behavior and protein constitution of all cells were subsequently characterized. In all cell types tested, cytotoxicity in control and resistant cells following exposure to OTA and OTB (nM - μ M), was assessed *via* the endpoints of MTT reduction (MTT), neutral red uptake (NR) and cell number analysis (CN).Using MTT or CN as endpoint, resistant cells displayed a cytotoxic response similar, but slower in onset to control cells. Interestingly, NR experiments showed that intracellular dye trapping was tremendously increased in resistant cells, suggesting these cells have undergone considerable changes with respect to an increase in acidic compartments and/ or altered membrane function. Our results support the aforementioned hypothesis that renal epithelial cells are fundamentally altered following chronic ochratoxin exposure.

399 SPECIES COMPARISON OF RENAL [^{3}H]OCHRATOXIN B PROTEIN BINDING AND UPTAKE *IN VITRO*.

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The mycotoxins ochratoxin A and B (OTA, OTB) are known food and feed contaminants produced by certain Aspergillus and Penicillium strains. Chronic dietary intake has been associated with increased incidences of Porcine Mycotoxic Nephropathy, (PMN) and also of urothelial tumors (UT) and nephropathies in humans (Balkan Endemic Nephropathy, BEN). OTA has also been shown to be carcinogenic in rodents with distinct species and sex-differences. OTB, which differs from OTA only in the substitution of an H-group for a Cl-group, has been shown to be significantly less toxic than OTA both *in vivo* and *in vitro*. This fact raises the question whether structure-based differential binding to certain protein structures and/or differential transport mechanisms play a role in ochratoxin toxicity. The aims of this study were to investigate protein binding and uptake of OTB in both sexes of relevant species for comparison with OTA. Such differences could at least in part explain the observed variability in toxicity. [^{3}H]OTB uptake was measured in primary and continuous renal lines from the same species. Similar uptake patterns were apparent as previously noted for [^{3}H]OTA. Protein binding to renal cortex tissue homogenates from both sexes of four species (human, pig, rat and mouse), was assessed *via* a [^{3}H]OTB competitive assay. Specific saturable binding of OTB was observed to be consistently lower than that of OTA in both capacity and affinity. The same binding rank order was determined for both substances : human > rat > pig > mouse. Substances which have been demonstrated to inhibit OTA protein binding in a concentration-dependent manner such as BSP, indomethacin and furosemide, also inhibited OTB binding, however competition patterns differed from those of OTA.

400 COMPARISON OF OCHRATOXIN BINDING TO HUMAN, PORCINE AND RAT RENAL PROTEINS *IN VITRO*.

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Ochratoxin A and B are found as contaminants in foodstuff and suspected of being one of the etiological agents responsible for Balkan endemic nephropathy (BEN) and urinary tract tumors. The dechloro- analogue, OTB is less toxic than OTA in animal studies, suggesting strict structure-activity requirements for toxicity. Ochratoxin A has previously been shown to bind to serum albumin and is also suspected to bind to cellular proteins of the kidney, which could be a prerequisite for toxicity. To detect these renal proteins, OTA and OTB were coupled covalently to horseradish peroxidase (HRP) and were used similarly to an antibody in Western Blot analysis. With this method it was investigated if variations in OTA and OTB binding to renal proteins could explain the observed structure- species- and sex- differences. Several proteins from rat, pig and human kidney as well as from proximal human kidney cells (HKC) were detected using OTA-HRP as staining method and displayed large intensity differences. Comparison of Ponceau S stained membranes with the signal obtained using OTA-HRP, showed that only a few of these proteins had an "above-average-binding" for OTA. This implies certain proteins to have a higher affinity for OTA binding than others.Proteins of approx. 33, 40, 63 kDa, which displayed this characteristic, were detected in all samples tested (male and female). Additional binding-proteins of 20 and 72kDa were detected in rat and human tissue respectively. HKC from both sexes displayed additional proteins of 105 and >250kDa. Similar, but weaker signals were detected with OTB. Addition of 15mM sulphobromophthalein (BSP), a substance previously shown to inhibit OTA binding, extinguished both the OTA-HRP and OTB-HRP signals. Phenylalanine or aspartame given separately at 15mM or in combination at 15mM, weakened the binding of both conjugates to human kidney proteins and to HKC but not to rat or porcine proteins.

401 DISRUPTION OF GLOMERULAR MESANGIAL AND VISCERAL EPITHELIAL CELL INTERACTIONS *IN VITRO* BY BENZO(A)PYRENE.

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Previous studies in this laboratory have shown that the adaptive response of rat glomerular cells to benzo(a)pyrene (BaP), a prototypical polycyclic aromatic hydrocarbon, is characterized by mesangial cell injury and unregulated proliferation of visceral epithelial cells (i.e. podocytes) *in vitro* and *in vivo*. These phenotypic profiles implicate disruption of critical glomerular mesenchymal/epithelial interactions following hydrocarbon challenge. To test this hypothesis, cytochrome (CYP) 1a1 and 1b1 gene expression and cellular proliferation in mesangial/podocyte co-cultures, or in response to conditioned medium, candidate mesenchymal-derived inhibitors of proliferation, including transforming growth factor beta 1 (TGFb1), prostaglandin E2 (PGE2), and nitric oxide (NO), and matrix cross plating experiments were conducted in clonal populations of mesangial cells (SCC1) and podocytes (SCC4E) challenged with 3μM BaP. Differential constitutive and inducible CYP gene expression and activities were observed in SCC1 and SCC4E treated with BaP, a pattern consistent with differences in susceptibility to BaP injury. SCC 4E proliferation was inhibited by SCC1 in direct and indirect co-culture. Denatured conditioned media also inhibited SCC4E proliferation, but no difference between control and BaP groups was observed. TGFb1 (0.01-0.5 nM), PGE2 (0.02-2μM) and NO (0.01-1 mM) did not influence SCC4E proliferation. Cross plating experiments demonstrated that the matrix deposited by SCC1 inhibited SCC4E proliferation. We conclude that disruption of mesenchymal/epithelial interactions by BaP may contribute to the loss of selective permeability induced by BaP *in vivo*. These alterations are reminiscent of the aberrant proliferation of podocytes observed in several forms of human glomerular disease. (Supported by ES 04917 and ES 09106).

402 SYSTEMIC UPTAKE OF INHALED ARSENIC IN RABBITS.

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Human occupational exposure to sufficiently high levels of arsenic in air has been associated with lung cancer, but generally not other types of cancer. Thus, a better understanding of the relationship between airborne arsenic exposures and systemic uptake is essential. In this study, rabbits were exposed to one of four levels of arsenic trioxide in air for eight weeks (0.05, 0.1, 0.22, or 1.1 mg/m^3). Plasma levels of inorganic arsenic, monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) were measured following the last exposure. Statistically significant increases in mean inorganic arsenic levels in plasma were observed only in male rabbits exposed to 0.22 mg/m^3, and in both males and females exposed to 1.1 mg/m^3. Mean inorganic arsenic levels in plasma in males and females exposed to 0.05 and 0.1 mg/m^3, and females exposed to 0.22 mg/m^3, were not significantly elevated compared to controls. These results suggest that arsenic inhalation has a negligible impact on body burden of inorganic arsenic until air levels are significantly elevated. Based on plasma measurements of inorganic arsenic, the two lowest exposure levels in this study (0.05 and 0.1 mg/m^3) are indistinguishable from background. These results have significant implications for risk assessments of inhaled arsenic. To the extent that an exposure has no discernible impact on body burden, attributing an incremental risk or directing risk management actions toward such an exposure would be inappropriate.

403 INORGANIC ARSENIC BIOTRANSFORMATION AND MITOCHONDRIAL TOXICITY IN HK-2 HUMAN PROXIMAL TUBULAR CELLS.

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Arsenic is an environmental toxicant and a human carcinogen. The kidney is a known target organ and is critical for both *in vivo* arsenic biotransformation and elimination. Thus the toxicity and metabolism of inorganic arsenic was investigated in an immortalized human proximal tubular epithelial cell line, HK-2. Subcytotoxic concentrations of arsenite (<10 μM) and arsenate (<100 μM) were determined by leakage of LDH from cells exposed for 24 hr. The threshold concentration of arsenite (1-10 μM) and arsenate (<25 μM) on the mitochondria was found by evaluating the metabolism of MTT. Biotransformation of inorganic arsenicals was determined in cells exposed to either arsenite or arsenate at the pre-determined subcytotoxic concentrations. Arsenic metabolites were detected in cell culture media and cell lysates using HPLC-ICP-MS, with a limit of detection of 0.5 ppb. No metabolites were detected in lysates or media following 6-hr exposures to either arsenical, indicating that metabolism of inorganic arsenic in these cells may be a slow event. A 24-hr exposure to arsenite resulted in minimal oxidation to arsenate in media (~5%), and only arsenite was detected in lysates. Exposure to arsenate resulted in its reduction to arsenite (~25%), with both inorganic metabolites detected in the media, and only arsenite detected in lysates. Pentavalent methylated arsenicals were not detected in media or lysates following exposure to either inorganic arsenical. However, the arsenic metabolites associated with the protein fraction of cell lysates or media have not been determined, and it is possible that some trivalent methylated metabolites may be present in that fraction. From these results it appears this human renal cell line is capable of biotransforming inorganic arsenic compounds, primarily reducing arsenate to arsenite. In addition, even at low concentrations, the mitochondria are a primary target for toxicity. (NIH ES04940)

404 DOSE-DEPENDENT BIOTRANSFORMATION OF ARSENITE IN RATS - NOT S-ADENOSYLMETHIONINE (SAM) DEPLETION IMPAIRS ARSENIC METHYLATION AT HIGH DOSE.

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Arsenite (AsIII) is eliminated by excretion and methylation. Monomethylarsonous acid (MMAsIII) is the most toxic metabolite of arsenic (As), while dimethylarsinic acid (DMAsV) produced in the next step is relatively atoxic. Methylation of As is carried out by SAM-dependent methyltransferases, which are inhibited by AsIII *in vitro*. Since the role of methylation in the toxicity and elimination of inorganic As is unclear, we have examined *in vivo* how excretion and tissue retention of AsIII and its metabolites change with increase of AsIII dose. Rats were injected iv with 20, 50, 125 μmol/kg AsIII and As metabolites in bile, urine and tissues were analyzed by HPLC-hydride generation-atomic fluorescence spectrometry. It was found that the excretion of AsIII *via* bile and urine and its tissue concentration increased proportionately or more to the dose, whereas the excretion and tissue concentrations of methylated metabolites (MMAsIII, monomethylarsonic acid, DMAsV) increased less than the dosage, or it even decreased after injection of the largest dose of AsIII. To elucidate the mechanism of the dose-dependent decrease of methylation, we dosed rats iv with 0, 20, 50, 125 μmol/kg AsIII and quantified SAM, S-adenosylhomocysteine (SAH), total glutathione (GSH), ATP, ADP and AMP in the liver 1 hr later. AsIII decreased in a dose-dependent manner the hepatic concentrations of GSH and ATP and the energy charge, but increased the level of SAM and SAH. Thus, the decrease of As methylation after injection of a large dose of AsIII cannot be due to SAM shortage, but may be caused by the concentration-dependent inhibition of the participating methyltransferases by AsIII. It appears that exhausted elimination capacity of AsIII, which results in AsIII retention in tissues, rather than MMAsIII produced from AsIII, is a main contributing factor in the acute toxicity of AsIII. After GSH depletion, the retained AsIII can increasingly inhibit SH-enzymes, thus causing ATP depletion and energetic disorder. (Supported by OTKA and the Hungarian Ministry of Health)

405 MITOCHONDRIA WORK AS REACTORS IN REDUCING ARSENATE TO ARSENITE.

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Arsenate (AsV) is a structural analogue of phosphate (Pi), yet its toxic effect is likely due to its reduction to the more toxic arsenite (AsIII). The cellular and molecular mechanisms of this process are still unclear. Since mitochondria take up AsV like Pi, we studied whether these organelles reduced AsV to AsIII, and if they did so, what the characteristics of this process were. Rat liver mitochondria were isolated and incubated with AsV. AsV and its metabolites were then quantified by HPLC-hydride generation-atomic fluorescence spectrometry analysis of the deproteinized incubates. We found that mitochondria rapidly reduced AsV. Among the substrates supporting the citric acid cycle glutamate enhanced the reduction most effectively. ADP increased, whereas AMP and ATP decreased the AsIII formation. These effects could be prevented by atractyloside, an inhibitor of adenine nucleotide translocator. Electron transport inhibitors (rotenone, antimycin A, azide) and uncouplers (CCCP, dicumarol) abolished AsIII formation. ATP synthase inhibitors (oligomycin, aurovertin B) strongly but incompletely inhibited AsIII formation. Phosphate decreased AsIII formation in a concentration-dependent manner. Inhibitors of mitochondrial Pi transporters (mersalyl, N-ethyl-maleimide and butylmalonate) abolished AsIII formation. Compounds transported by the also Pi-moving dicarboxylate carrier (sulfate, sulfite and thiosulfate) caused partial inhibition. AsIII was recovered completely from the supernatant of the mitochondrial incubate, suggesting that mitochondria exported all of the formed AsIII. Upon solubilization of mitochondria their AsV reducing activity was practically lost. Summarizing, our findings support the view that mitochondria work as reactors: they take up AsV, rapidly reduce it to AsIII and export this metabolite. Chemical impairment of the mitochondrial function or disruption of structure inhibits the biotransformation of AsV into AsIII in this organelle. (Supported by OTKA and the Hungarian Ministry of Health)

406 RAT LIVER CYTOSOL REDUCES ARSENATE TO ARSENITE IN THIOL- AND PURINE NUCLEOSIDE-DEPENDENT MANNER.

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As liver mitochondria reduce arsenate (AsV) to the more toxic arsenite (AsIII) (see the poster of Schweibert, Németi and Gregus), we examined if other cell fractions also carried out this process. Postmitochondrial supernatant (PMSN) was isolated from rat liver and incubated with AsV. AsV and its metabolites were analyzed by HPLC-HG-AFS. We observed that PMSN reduced AsV to AsIII only in the presence of a thiol. Dithiothreitol (DTT) supported the reduction much more effectively than glutathione. Membrane solubilising detergents enhanced AsIII formation. PMSN was separated into microsome and cytosol and AsV reducing activities of these fractions were measured. Only the cytosol reduced AsV. Phosphate (Pi) inhibited the cytosolic AsV reducing activity in a concentration-dependent manner. Mercurial thiol reagents also inhibited the process, indicating the involvement of a Pi-utilizing SH-enzyme. On searching for a reduction partner, it was found unexpectedly that oxidized pyridine nucleotides (NAD or NADP), but not their reduced forms, increased AsIII formation. Some other purine nucleotide derivatives, but not pyrimidine nucleotides, also increased the rate 2-3 fold. Examination of the effect of nucleosides and nucleobases on AsV reduction yielded dramatic results: purine nucleosides (inosine or guanosine) increased the rate 80-100 fold, whereas purine bases (hypoxanthine or guanine) decreased it 85-95%. Although the retentate obtained by ultrafiltration of cytosol was inactive, its AsV reductase activity could be regained by adding the filtrate or inosine or guanosine to the retentate, indicating that endogenous purine nucleosides were essential for AsV reduction by the cytosol. Thus, rat liver cytosol is able to reduce AsV to AsIII. The reduction requires the presence of an appropriate thiol as well as a purine nucleoside (inosine or

guanosine) and is inhibited by thiol reagents, the AsV analogue phosphate and purine bases. Identification of the cytosolic AsV reductase is presented in the accompanying poster (Gregus and Németi). (Supported by OTKA and the Hungarian Ministry of Health)

407 PURINE NUCLEOSIDE PHOSPHORYLASE (PNP) AS A CYTOSOLIC ARSENATE REDUCTASE.

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The findings of the accompanying poster (Németi and Gregus) indicated that the arsenate reductase (AsVR) activity of rat liver cytosol is due to an SH-enzyme that uses phosphate (or its analogue arsenate, AsV) and a purine nucleoside (guanosine or inosine) as substrates. Such an enzyme is PNP that catalyses the phosphorolytic cleavage of 6-oxopurine nucleosides according to the following scheme: guanosine (or inosine) + phosphate « guanine (or hypoxanthine) + ribose-1-phosphate. Therefore, we tested the hypothesis that PNP is responsible for the thiol- and purine nucleoside-dependent reduction of AsV to AsIII by rat liver cytosol. AsIII formed from AsV was quantified by HPLC-hydride generation-atomic fluorescence spectrometry analysis of the deproteinized incubates. The following findings support the conclusion that PNP reduces AsV to AsIII, using AsV instead of phosphate in the reaction above: 1. Specific PNP inhibitors (CI-1000, BCX-1777) at a concentration of 1 μM completely inhibited cytosolic AsVR activity. 2. During anion-exchange chromatography of cytosolic proteins PNP activity perfectly coeluted with the AsVR activitiy, suggesting that both activities belong to the same protein. 3. PNP purified from beef liver catalyzed reduction of AsV to AsIII in the presence of dithiothreitol (DTT) and a 6-oxopurine nucleoside (guanosine or inosine). 4. AsVR activity of purified PNP (like cytosolic AsVR activity, see Németi and Gregus) was inhibited by phosphate (the substrate of PNP competing with AsV), guanine and hypoxanthine (products of PNP favoring the reverse reaction), mercurial thiol reagents (non-specific inhibitors of PNP) as well as CI-1000 and BCX-1777 (specific PNP inhibitors). Thus, PNP is responsible for the AsVR activity of rat liver cytosol in the presence of DTT. Further research should clarify the mechanism and the *in vivo* significance of PNP-catalyzed reduction of AsV to AsIII. (Supported by OTKA and the Hungarian Ministry of Health)

408 ARSENIC INDUCES ABERRANT GENE EXPRESSION DURING LUNG DEVELOPMENT: APPLICATION OF TOXICOGENOMICS.

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Arsenic is a known human carcinogen and exposure has been correlated with an increased incidence of lung cancers. Such studies strongly suggest that the lung is directly targeted by arsenic. Those exposed to arsenic through contaminated food and drinking water include pregnant women, with unknown developmental consequences in the fetal lung. We hypothesize that *in utero* exposure to inorganic arsenic causes altered gene expression leading to molecular and physiological changes in the lung. Pregnant Sprague-Dawley rats were exposed to 500 ppb sodium arsenite in the drinking water, from embryonic days one to eighteen. Subtractive hybridization of embryonic lung cDNA from control and treated day 18 embryos yielded 248 differentially expressed cDNA clones. Of 154 clones screened thus far, 93 are confirmed differentially expressed by Southern dot blot hybridization and have been sequenced. Dose and time dependent effects of arsenite on gene expression patterns in developing rat lung will be evaluated using a custom cDNA microarray, including the 248 clones from the subtractive hybridization. This custom microarray is also to include an additional 112 rat cDNA clones. Clones showing differential expression by cDNA microarray will be sequenced, and the expression of selected clones will be validated using Northern blotting and PCR. The effects of altered gene expression on lung development will then be assessed using specific probes to show localization of gene and protein expression. (Supported in part by the NIEHS Superfund Basic Research Program Grant P42 ES04940).

409 GENE EXPRESSION PROFILE OF ARSENITE-INDUCED SKIN TOXICITY: MECHANISTIC IMPLICATIONS FOR CARCINOGENICITY.

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Human exposure to arsenic is associated with an increased incidence of cancer, in particular, skin cancer; however, mechanism(s) associated with AsIII-mediated toxicity and carcinogenesis at low levels of exposure remain elusive. Modulations in cell proliferation, oxidative damage, and DNA-repair fidelity have been implicated in AsIII-mediated toxicity, but the concomitant occurrence of these major events has not been demonstrated in response to arsenic exposure. We hypothesized the involvement of these alterations in arsenic-mediated human toxicity and investigated the time- and dose-dependent effects of arsenite (0.005-5μM, 1-24hr) in normal diploid human epidermal keratinocytes by performing cDNA microarray analysis to elucidate AsIII-mediated global gene expression changes. In addition, we examined biochemical (DNA synthesis) and physiological (cellular mitoses) changes in the proliferative response of NHEK to AsIII exposure in order to better correlate alterations in gene expression with cellular function. Our results indicate that AsIII exposure decreased transcripts associated with DNA repair (e.g. p53 and MSH5), and increased transcripts indicative of cellular response to oxidative stress (e.g. SOD1 and NAD(P)H oxidoreductase). AsIII modulated the expression of transcripts indicative of increased cell proliferation (e.g. FGFR4 and PKC), oncogenes, and genes associated with cellular transformation (e.g. Gro-1 and V-yes). These observations correlated well with DNA synthesis and mitotic measurements as AsIII treatment resulted in a dose-dependent increase in cellular mitoses at 24 hr and an increase in DNA synthesis at 48 hrs of exposure. The data presented here shows that arsenic results in gene expression indicative of decreased DNA repair, increased cell proliferation and oxidative stress. Many of the gene expression alterations reported here are indicative of novel pathways perturbed by AsIII which we hypothesize to play a major role in its mechanism of carcinogenesis.

410 ACTIVATION OF AP-1 IN UROTSA CELLS BY METHYLATED TRIVALENT ARSENICALS.

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Chronic exposures to inorganic arsenic (iAs) increase risks of various types of cancer in humans. However, mechanisms by which iAs causes cancer are unknown. Biomethylation is the major pathway for iAs metabolism in humans. Both trivalent and pentavalent iAs and methylated arsenicals are intermediates or final products in this pathway: iAsV -> iAsIII -> MAsV -> MAsIII -> DMAsV -> DMAsIII. Recent reports have shown trivalent methylated metabolites, methylarsonous acid (MAsIII) and dimethylarsinous acid (DMAsIII), to be more cytotoxic, genotoxic and more potent enzyme inhibitors than either iAsV or iAsIII, indicating that methylation increases toxic and carcinogenic potency of iAs. To further characterize the role of trivalent methylated metabolites in the toxicity and carcinogenicity of iAs, we have examined effects of iAsIII, MAsIII and DMAsIII on nuclear levels of proto-oncogenes c-jun and c-fos and on the activation of AP-1 in UROtsa cells, an SV-40 immortalized human urothelial cell line. Short-time (0.5 to 2 h) exposures to 0.1 to 5 μM iAsIII, MAsIII or DMAsIII significantly increased nuclear levels of p-c-jun and DNA-binding activity of AP-1. Western blot and EMSA/supershift analyses indicated that c-fos was not involved in activation of AP-1 by these arsenicals. Notably, MAsIII and DMAsIII were considerably more potent inducers of c-jun phosphorylation and AP-1 activation in UROtsa cells than was iAsIII. Western blot analyses of the MAPK enzyme family in cells exposed to trivalent arsenicals showed a significant increase in p-ERK but not in p-JNK or in p-p38 levels. The increase in p-ERK levels was more profound in cells exposed to MAsIII and DMAsIII and correlated with increased levels of p-c-jun. These results indicate that MAsIII and DMAsIII, toxic intermediates of arsenic metabolism, activate AP-1 in human urothelium and affect AP-1-dependent gene transcription. (This abstract does not necessarily represent policy of the USEPA.)

411 CARCINOGENICITY OF INORGANIC ARSENIC IN MICE: TUMORS AND PROLIFERATIVE LESIONS OF THE LIVER, ADRENAL, OVARY, UTERUS AND OVIDUCT IN MICE EXPOSED TO SODIUM ARSENITE DURING GESTATION.

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Arsenic (As) is a known human carcinogen, but development of rodent models of inorganic As carcinogenesis has been problematic. Since gestation can be a period of high sensitivity to chemical carcinogenesis, we performed a transplacental carcinogenicity study in mice with inorganic As. Groups (n = 10) of pregnant C3H mice received drinking water containing As (as sodium arsenite; $NaAsO_2$) at 0 (control), 42.5 and 85 ppm As *ad libitum* from day 8 to18 of gestation. These doses where well tolerated. Dams were allowed to give birth, and offspring were weaned at 4 weeks and then randomly put into separate groups (n = 25) of males and females according to maternal dose. Offspring were observed for up to 104 weeks. A complete necropsy was performed on all mice and tissues were microscopically examined. In male offspring, there was a marked, dose related increase in hepatocellular carci-

noma incidence (control, 3 carcinoma/24 mice at risk; 42.5 ppm, 8/21; 85 ppm, 14/23) and in liver tumor multiplicity (tumors/organ; 5.6-fold over control at 85 ppm). A dose related increase in adrenal tumor incidence (control, 9/24; 42.5 ppm, 14/21; 85 ppm, 21/23) and multiplicity (2.2-fold over control at 85 ppm) and a dose related trend for increased lung tumor incidence also occurred in males. In females, dose-related increases occurred in incidence of ovarian tumors (control, 2/25; 42.5 ppm, 6/23; 85 ppm, 9/24), uterine proliferative lesions (moderate to severe cystic hyperplasia plus tumors; control, 4/25; 42.5 ppm, 13/23; 85 ppm, 15/24) and hyperplasia of the oviduct (control, 0/25; 42.5 ppm, 3/23; 85 ppm, 6/24). A dose related trend also occurred for increased lung tumor incidence. This study shows that inorganic As, as a single agent, induces tumors at multiple sites after transplacental exposure, and establishes inorganic As as a complete carcinogen in mice. The liver and lung are both target sites of As carcinogenesis in humans.

412 DIFFERENTIAL SENSITIVITY OF HUMAN AND MURINE KERATINOCYTES TO SODIUM ARSENITE: EFFECTS ON CELLULAR VIABILITY AND PROLIFERATION.

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Arsenic is a toxicant and carcinogen that poses a significant health risk in humans. Although the mechanisms contributing to arsenic-induced skin cancer are not completely understood, the generation of oxidative stress is thought to be central to the carcinogenicity of this agent. Based on evidence that arsenic has different biological effects on human, mouse, normal and transformed cells, this study addressed the hypothesis that arsenic-induced toxicity and cell proliferation are differentially modulated in normal human epidermal keratinocytes (NHEK) and immortalized keratinocytes (human HaCaT and mouse HEL30). To test this hypothesis, cellular viability was determined by quantifying neutral red uptake, and cell proliferation quantified by measuring [3H]-thymidine incorporation into DNA following short-term sodium arsenite exposure. The effect of glutathione depletion/enhancement on viability following arsenite exposure also was evaluated by addition of L-buthionine-[s, r]-sulfoximine (BSO) or *N*-acetyl cysteine (NAC) to culture media. Results indicate the LC_{50} for arsenite in NHEK, HaCaT, and HEL30 keratinocytes is approximately 5-10 μM. 3 μM arsenite enhances DNA synthesis > 100% in NHEK as compared to control (no arsenite) but has little effect on immortalized keratinocyte DNA synthesis. BSO increases arsenite-mediated cytotoxicity by reducing the LC_{50} to approximately 1-3 μM in all cell types examined, whereas NAC attenuates the viability enhancing effects of low concentrations of arsenite in NHEK. These data suggest that endogenous glutathione modulates the negative and positive effects of arsenite on cellular viability. Taken together, these findings indicate that normal human and immortalized human/mouse keratinocytes have differential sensitivities to arsenite-induced DNA synthesis but similar cytotoxicity profiles. These data further suggest that NHEK may provide an accurate *in vitro* model in which to study the role of oxidative stress in arsenic-induced skin cancer.

413 CHRONIC INORGANIC ARSENIC EXPOSURE IN MICE INDUCES HEPATIC GLOBAL DNA HYPOMETHYLATION AND OVER EXPRESSION OF THE ESTROGEN RECEPTOR GENE ALONG WITH REDUCED METHYLATION OF ITS PROMOTER REGION.

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Inorganic arsenic (As) is a human carcinogen that targets the liver, but its mechanism is unknown. Many species, including humans, methylate As, consuming cellular methyl groups, which may perturb normal methylation. We find global DNA hypomethylation occurs during As-induced malignant transformation *in vitro* in rodent liver cells. DNA hypomethylation can increase gene expression, particularly when occurring in promoter region CpG sites, and may be a non-genotoxic mechanism of carcinogenesis. Thus, we extended our work to an *in vivo* system by exposing 129/SV male mice (6/group) to 0 or 37.5 ppm As (as $NaAsO_2$) in the drinking water for 48 weeks. Liver samples were examined for global DNA methylation, generalized aberrant gene expression, specific gene expression, and specific gene promoter region methylation. Exposure to As induced global DNA hypomethylation, as assessed by 5-methylcytosine content or methyl acceptance assay. Microarray analysis showed As induced a 2.5-fold increase in estrogen receptor-α (*ER*) gene expression. RT-PCR and immunohistochemistry confirmed As-induced increases in hepatic *ER* transcript and translation product, respectively. Estrogens can be carcinogenic, with tissue sensitivity linked to *ER* expression, and *ER* expression can be controlled by methylation. When the 13 CpG sites in the *ER* gene promoter region from hepatic DNA were examined by methylation specific PCR, all showed less methylation after As, and this was significant ($p < 0.01$) in 8/13 sites. Overall, 28.3% of the *ER* promoter region CpG sites were methylated in controls, but only 3.2% were methylated after As exposure. Thus, chronic As exposure *in vivo* can induce global DNA hypomethylation and hypomethylation of the promoter region of the *ER* gene. This likely increases *ER* expression and, as estrogens can be hepatocarcinogens, may be an early event in As carcinogenesis, at least in the liver.

414 ARSENIC IS AN ENDOCRINE DISRUPTOR, BLOCKING HORMONE-MEDIATED GENE REGULATION BY THE ESTROGEN AND GLUCOCORTICOID RECEPTORS *IN VIVO*.

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Arsenic (As) is an environmental agent of considerable human health concern in the US and around the world. Exposure to elevated drinking water As has been associated with increased risk of several cancers, vascular disease, diabetes, and reproductive / developmental problems. These risks have been shown to be elevated in populations with drinking water arsenic in the range near the current USEPA drinking water standard found in many areas of the US We previously reported that As(III) can act as an endocrine disruptor, almost completely blocking hormone-mediated activation of gene expression by the glucocorticoid receptor (GR) in cultured H4IIE rat hepatoma cells. We investigated these effects further in the current work. At the cell physiological level, As was shown to block GR hormone-mediated control of H4IIE cell growth. As was found to bind preferentially and nearly stoichiometrically to GR at extremely low (nM) doses using $^{73}As(III)$. Using various GR mutants in a GR deficient H4IIE-derived cell line, we also observed that the effects of As on GR signaling most closely mapped to the central DNA-binding domain of GR, which is highly conserved among steroid hormone receptors in the nuclear receptor superfamily. Based on this, we then examined whether As would disrupt estrogen receptor (ER)-mediated gene regulation. In cultured H4IIE rat hepatoma cells, 0.1-3 μM As(III) (4-18 hr) supressed estradiol induction of both native and artificial ER binding element (ERE)-containing genes. As(III) at non-overtly toxic doses (50-100 μmol/kg) also strongly suppressed ER-mediated estradiol induction of vitellogenin gene expression in 14 day chick embryo liver *in vivo*. These results indicate that As(III) targets both GR and ER, and possibly other steroid receptors, at doses that are directly relevant to elevated As levels in US drinking water. (NIEHS (SBRP) ES07373)

415 ARSENITE INDUCES MITOTIC ARREST AND APOPTOSIS IN SV40-TRANSFORMED HUMAN SKIN FIBROBLASTS.

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Chronic arsenic ingestion causes skin, bladder and liver cancer by an unknown mechanism. Arsenite is known to delay transit through M-phase in normal diploid human fibroblasts (NDHF). Compared to cycling or quiescent NDHF, continuously cycling SV40-transformed human fibroblasts (STHF) were observed to be hypersensitive to arsenite toxicity and the mechanism of this hypersensitivity was investigated. Five - 20 μM $NaAsO_2$ induced cytostasis in cycling NDHF but was not obviously toxic to non-cycling NDHF. Higher concentrations were overtly cytotoxic to both cycling and quiescent NDHF. Concentrations as low as 3 μM were cytotoxic to STHF. Phase microscopy of $NaAsO_2$-treated STHF suggested concentration-dependent accumulation of cells in mitosis undergoing apoptosis. Toxicity measured using AlamarBlue indicated the $NaAsO_2$ LD50 was 24.1 μM in cycling NDHF and 3.5 - 4.3 μM in the two STHF lines tested. Arsenite treatment of STHF with 0 - 10 μM $NaAsO_2$ induced concentration-dependent inhibition of cell proliferation, accumulation of cells with G2/M DNA content, and increased mitotic index. Induction of apoptosis was indicated by membrane blebbing observed by phase microscopy, by annexin V binding and by electron microscopy showing highly vacuolated cells containing chromosomes surrounded by intact blebbed cellular membranes. These results indicate that SV40 transformation sensitizes cells to arsenite induced mitotic arrest and induction of apoptosis in the mitotic cells. Supported NIH grants R25 RR12242, P30ES06639, R01 ES06460, R01 ES09392 and Commonwealth of Kentucky Research Challenge Trust.

416 TOXICITY OF PENTAVALENT AND TRIVALENT ARSENICALS IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS: ROLE OF PH.

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Chronic exposure to low levels of arsenic (As) in the environment has been associated with disorders of vascular endothelial cells (ECs) and a variety of cancers, including hepatic angiosarcoma, in which the cell of origin is the hepatic sinusoidal EC. The mechanisms underlying As-induced EC dysfunction and its carcinogenic effects remain unresolved. Under both physiological and pathological conditions, ECs can be exposed to an acidotic microenvironment, which is known to influence the progression of many diseases, including cancer. To investigate whether an acidotic microenvironment mediates the toxic effects of As in ECs, we evaluated, under conditions of normal physiological pH and acidosis, the toxicities of trivalent and pentavalent inorganic As and pentavalent methylated As metabolites. Human umbilical vein endothelial cells (HUVECs) were exposed to arsenate [As(V)], arsenite [As(III)], methylarsenate [MAs(V)], or dimethylarsinic acid [DMAs(V)] at concentrations in the milli- and micromolar ranges for 24 hours under conditions of 5% CO_2 or 20% CO_2, to achieve a culture medium pH of 7.4 or 7.0, respectively. HUVEC viability was assessed using the MTT assay. Preliminary results indicate that at pH 7.4, As(III), with an LC_{50} of 30 μM, was more toxic to HUVECs than As(V), MAs(V), or DMAs(V), with LC_{50}s of 7, 25, and 50 mM, respectively. At pH 7.0, the LC_{50}s of As(V), MAs(V), and DMAs(V) were similar to those at pH 7.4 (10, 15, and 75 mM, respectively). However, at pH 7.0, the LC_{50} of As(III) was 125 μM, approximately four times higher than at pH 7.4. These results suggest that acidosis may modulate the toxicity of trivalent As species in HUVECs. Evaluation of the toxicity of trivalent methylated As metabolites is also planned. This project is funded by the NCI (K08 CA72396-05) and ATSDR(U61/AT11881475).

417 POTENTIAL CELLULAR MECHANISMS FOR ARSENITE-INDUCED VASCULAR DISEASES.

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Chronic, low level exposure to arsenite increases the incidence of proliferative vascular diseases, such as arteriosclerosis and ischemic heart disease. Arsenic-associated changes in blood vessels may contribute to the vascular components of diabetes and tumor growth. Angiogenesis is fundamental to most of these pathological changes and may underlie the vascular actions of arsenic. In support of this hypothesis, arsenite (0.033-1.0 μM) significantly increased blood vessel growth in an *in vivo* chicken embryo model. Above 1.0 μM, arsenite inhibited blood vessel growth. To examine potential mechanisms for these vascular effects, expression of the angiogenic genes, vascular endothelial cell growth factor (VEGF) and plasminogen activator inhibitor-1 (PAI-1), was examined in porcine aortic smooth muscle cell (SMC) cultures. Concentrations of up to 50 μM arsenite caused SMC to proliferate. Release of VEGF protein increased following a 24 h exposure to 5-25 μM arsenite. By 48 h, 1 μM arsenite caused significant VEGF release. Dose and time dependent changes in VEGF and PAI-1 mRNA levels were consistent with the changes in protein levels. However, VEGF expression continued to increase above 5 μM arsenite, while PAI-1 mRNA levels peaked at 2.5 μM arsenite and dropped to control levels by 10 μM. Arsenite levels increased hypoxia inducible factor (HIF) protein levels in a manner consistent with enhancement of the VEGF and PAI-1 promoter activity. Inhibitor studies demonstrated that arsenite stimulated oxidant formation and kinase cascades to increase HIF levels and angiogenic gene expression. Microarray analysis in human airway epithelial cells, demonstrated that exposure to arsenite (5 or 50 μM) for 4 h increased HIF mRNA levels by 1.8-3 fold. These data suggest that arsenite causes specific, dose-dependent effects on cell signaling that promote angiogenic responses, which contributes to pathologic vascular changes. *Supported by ES07373.*

418 EVALUATION OF *IN VIVO* ACUTE IMMUNOTOXICITY OF AN INORGANIC ARSENICAL, SODIUM ARSENITE, USING MURINE MODELS.

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It has been well known that inorganic arsenicals are potent toxicans and carcinogens in humans and most animals. However, immunotoxic effects of them have not been well studied. In this study, we observed *in vivo* acute immunotoxicity of an inorganic arsenical, arsenite, using murine models. Sodium arsenite was administered intraperitoneally to male CDF1 mice aged 4 weeks at doses of 1.25-8.0 mg/kg (body weight) on day 0 and multiple immune functions of these mice were assessed until day 16. As results, a half numbers of mice were died when 8 mg/kg of arsenite were administered. The mice body weights were decreased significantly by arsenite in a dose dependent manner at doses of 1.25-4.0 mg/kg on days 1-2, and it returned to the control level on day 4. Administered arsenite was distributed to various organs, including spleen, thymus, liver, lung, kidney and heart, with similar kinetic changes determined by AAS; the maximum arsenic concentrations were detected on day 1 and they dropped slowly to the control level during 16 days. The weights of immune organs, spleen, thymus and liver, were significantly decreased by arsenite at doses of 1.25-4 mg/kg on days 1-2, and they returned to the control levels on day 4. However, it did not affect on lung, kidney, heart and Peyer's patch weights. 4-mg/kg arsenite also decreased significantly the numbers of splenocytes, thymocytes, peritoneal exude cells, peripheral blood leukocytes and erythrocytes of mice temporary on day 1, but it did not affect on alveolar macrophage and Peyer's patch lymphocyte numbers. Cellular lysosomal enzyme activity and nitric oxide production of peritoneal macrophages, and blatogenesis of splenocytes and thymocytes were inhibited by 4-mg/kg arsenite on day 1 although the functions of alveolar macrophages, Peyer's patch lymphocytes and bone marrow cells were not changed. Taken together, arsenite has significant *in vivo* immunotoxicity both quantitatively and qualitatively, and the immune organs are very sensitive to arsenic inhalations.

419 ARSENIC-INDUCED SELF-TOLERANCE AFTER CHRONIC EXPOSURE IN HUMAN PROSTATE EPITHELIAL CELLS.

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While acquired self-tolerance to arsenic (As) has been shown in many rodent cell lines, acquisition of As resistance in human cells appears difficult to induce. Acquired As tolerance is linked to As efflux, probably mediated by increases in glutathione (GSH) levels, glutathione S-tranferase (GST) activity and the participation of efflux pumps such as MRP and MDR. Thus, we studied chronic As exposure of a human prostate cell line (RWPE-1) and development of As tolerance. RWPE-1 cells were continuously exposed to 0 or 5 μM arsenite (As^{+3}) for 18 or more weeks. Acute toxicity of As^{+3} (0-100 μM; 48 h) was then assessed using the MTS assay. The acute LC_{50} of As^{+3} in chronic As exposed prostate epithelial (CASE-PE) cells was 43.8 ± 2.3 μM *versus* 17.6 ± 2.9 μM in control cells, a 2.5-fold increase. CASE-PE cells grown in As-free medium for 5 weeks retained As-resistance, indicating a stable change in phenotype. As accumulation over 24 h in cells exposed to 10 μM As^{+3} was markedly reduced in CASE-PE cells (5.4 ng As/10^6 cells) compared to control cells (66.6 ng As/10^6 cells). When As-loaded cells were placed in As-free media for 2 h, the rate of As efflux was 2.6-fold greater in CASE-PE cells than control. CASE-PE cells showed increased GSH levels (4.9-fold) and increased GST activity (1.4-fold). GSH depletion with buthionine sulfoximine or inhibition of GST with ethacrynic acid both abolished As tolerance in CASE-PE cells. Although As-tolerant cells did not show changes in *MRP* or *MDR* gene expression at the transcription level, probenecid and PSC833 (specific inhibitors of MRP and MDR, respectively) both abolished As-tolerance. Thus, human prostate epithelial cells chronically exposed to As acquire tolerance to As. The mechanism of this tolerance appears to involve increases in GSH levels and GST activity, but not increases in *MRP* or *MDR* gene expression, although the functionality of these pumps is key to maintaining resistance.

420 IS THERE A SPECIAL SENSITIVITY OF CHILDREN TO INORGANIC ARSENIC?

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For some chemical exposures, the period of early childhood represents a stage of development characterized by increased sensitivity. There has been recent interest in the possibility of enhanced child susceptibility to the toxic effects of arsenic. This work evaluated the available evidence addressing this concern. While metabolic differences between children and adults have been noted, the evidence is contradictory. For example, while a study in Northern Argentina observed some differences between children and adults, a study of children in Washington State did not observe age-related differences in arsenic metabolite patterns. Studies of the health effects of arsenic in children *versus* adults provide a more relevant basis for discerning age-related differences in susceptibility. In a Chilean population of children exposed to high levels of arsenic, dermal effects (i.e., hyperkeratosis, leuko-melanoderma) were associated with an estimated daily arsenic intake of 0.063 mg/kg-day. This dose-effect level lies within the dose range derived from studies reporting similar effects in adults, i.e., 0.01 to 0.1 mg/kg-day. Other findings of specific health effects in children, including hearing changes and neurological deficits, are inconsistent. These studies also have limitations which preclude determining a definitive role for

arsenic. Thus, the available data do not clearly demonstrate children have a special sensitivity to the toxicity of arsenic, particularly at the low levels of arsenic exposure typically found in the US.

421 THE PROTECTIVE EFFECTS OF ASCORBIC ACID AND FOLIC ACID ON ACUTE ARSENIC TOXICITY IN CULTURED MOUSE FIBROBLASTS.

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The acute cytotoxic effects of arsenic can be modulated by a number of factors, including folic acid supplementation, glutathione status, and prior exposure to this metalloid. Based on the hypothesis that protective factors reverse arsenic-induced oxidative stress and/or alterations in cellular methyl metabolism, this study was designed to determine if the nutritional factors ascorbic acid (AA) and folic acid (FA) protect cultured cells against arsenic toxicity. Using the neutral red cytotoxicity assay, it was found that the antioxidant ascorbic acid at 10 and 30 μM significantly decreases the toxicity of 3 and 6 μM sodium arsenite (As) in C3H10T1/2 mouse fibroblasts. To determine if arsenic toxicity is influenced by the folic acid transporter Folbp2, primary fibroblast cell lines were created from Folbp2 +/+, +/-, and -/- embryos; the genotype of each cell type was corroborated by PCR screening. When exposed to As for 24 hours, Folbp2 null fibroblasts exhibit slightly more cytotoxicity than do wild type or heterozygous fibroblasts. The addition of 30 μM FA to Folbp2 null cells did not afford protection against arsenic toxicity. In contrast, the toxicity of sub-lethal concentrations of As was attenuated by FA in wild type cells. These results corroborate our earlier finding that FA protects SWV/Fnn fibroblasts from acute arsenic toxicity. In addition, it appears that this protective effect is dependent upon a functional Folbp2 gene. Because Folbp2 null mice exhibit an increased susceptibility to arsenic-induced neural tube defects, it is proposed that compromised Folbp2 function is a risk factor for arsenic-dependent toxicity secondary to compromised uptake of FA. Because exposure through drinking water and food is a major problem in many countries whose populations commonly are deficient in FA and AA, it is proposed that supplementation of these two nutrients might protect against As toxicity.

422 HEPATOPROTECTIVE EFFECTS OF *SEMISULCOSPIRA LIBERTINA* AND GARLIC ON THE LIVER DAMAGE INDUCED BY CARBON TETRACHLORIDE IN RATS.

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Semisulcospira libertina (Korean freshwater snail) has been used as a folk medicine for the treatment of various diseases such as jaundice, stomachache, dyspepsia, and diarrhea in the Eastern Asia. Also, garlic has many medicinal properties. This study was designed to investigate the hepatoprotective effects of *S. libertina* and garlic on the acute hepatotoxicity of carbon tetrachloride (CCl_4). Male Sprague-Dawley rats weighing 200-220 g were pretreated with dehydrated powder of *S. libertina* (2.1 g/kg, *po*; SL) and dehydrated powder mixture of *S. libertina* and garlic (3 g/kg, 7:3 ratio, *po*; SG) once daily for 3 consecutive days, given a single toxic dose of CCl_4 (1 g/kg in 5 ml/kg corn oil, *po*) and liver function determined 24 hr later. Liver damage was assessed by quantitating serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH), and alkaline phosphatase (ALP) activities as well as by histopathological examination. Pretreatments with SL and SG significantly decreased CCl_4-elevated ALT (48 %, 61 % respectively), AST (32 %, 47 %), and SDH (51 %, 76 %) activities, but had no effect on ALP. SL and SG have revealed hepatoprotective effects against CCl_4-induced histopathological changes such as severe necrosis, inflammatory cell infiltration and congestion in the central zone of hepatic lobule. SG enhanced the hepatic content of glutathione by 106 % compared with CCl_4 group, but SL increased to a somewhat lesser extent (65 %). These findings demonstrate that SL and SG have the hepatoprotective effect on CCl_4-induced liver damage.

423 HEPATOPROTECTIVE EFFECTS OF *SEMISULCOSPIRA LIBERTINA* AND GARLIC ON ACETAMINOPHEN-INDUCED LIVER DAMAGE IN MICE.

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Semisulcospira libertina (Korean freshwater snail) has been used as a traditional drug for the therapy of various diseases such as jaundice, stomachache, dyspepsia, and diarrhea in the Eastern Asia. Since the legendary medicinal properties, garlic has attracted scientific interests for several decades. The purpose of this study was to investigate the hepatoprotective effects of *S. libertina* and garlic on liver damage induced by acetaminophen (AA). Male ICR mice weighing 30-32 g were pretreated with dehydrated powder of *S. libertina* (3.5 g/kg, *po*; SL) and dehydrated powder mixture of *S. libertina* and garlic (5 g/kg, 7:3 ratio, *po*; SG) once daily for three consecutive days, given a single toxic dose of AA (450 mg/kg in saline, *ip*) and liver function determined 18 hr later. Liver damage was assessed by quantitating serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and sorbitol dehydrogenase (SDH) activities as well as by histopathological examination. Pretreatments with SL and SG dramatically lowered AA-elevated ALT (94 %, 97 % respectively), AST (88 %, 89 %), and SDH (93 %, 93 %) activities. SL and SG have revealed hepatoprotective effects against AA-induced histopathological changes. SG enhanced the hepatic content of glutathione by 43% compared with control animals. The gene expression of TNFα mRNA on AA-induced liver damage was determined by RT-PCR. SL and SG markedly decreased the gene expression of TNFα mRNA induced by AA in mice. These findings demonstrate that SL and SG have the hepatoprotective effect on AA-induced liver damage.

424 EXTRACT OF *ASTRAGALI RADIX* DECREASES ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN MICE.

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Astragali radix (AR) is one of the oldest and most frequently used crude drug for traditional medicine in many Asian countries. This study designed to investigate the hepatoprotective effects of the hot water-extracted AR against acetaminophen (AA)-induced hepatic damage in ICR mice. AA at the dose of 450 mg/kg *ip* produced liver damage in ICR mice. Pretreatment with AR significantly decreased serum enzyme activities of alanine aminotransferase, aspartate aminotransferase, and sorbitol dehydrogenase. However, concentration of hepatic glutathione did not show a significant change between the tested groups. We also investigated TNFα mRNA gene expression on AA-induced liver damage by RT-PCR. AA drastically induced TNFα mRNA gene expression in ICR mice. Pretreatment of mice with AR led to a marked decrease of TNFα mRNA gene expression. These data indicate that the protective effect of AR against AA-induced hepatotoxicity in mice appears to be related to the regulation of TNFα mRNA gene expression as well as biochemical alterations.

425 OLEANOLIC ACID INCREASES INOS AND TNF-A GENE EXPRESSION IN MACROPHAGES BY NF-KB ACTIVATION.

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Oleanolic acid (OA), a pentacyclic triterpene acid, is reported to have anti-tumor activities; however, the mechanism underlying its anti-tumorigenic effects is poorly understood. To further determine the mechanism of OA, we investigated the effects of OA on the release of NO and TNF-a, and on the level of iNOS and TNF-a gene expression in mouse macrophages. We found that OA elicited a dose-dependent increase in NO and TNF-a production. Reverse-transcription polymerase chain reaction showed that the increased NO and TNF-a secretion were due to an increase in iNOS mRNA and TNF-a mRNA, respectively. Since iNOS and TNF-a transcription has recently been shown to be under the control of the NF-kB transcription factor, the effects of OA on NF-kB activation were examined using a transient transfection assay and an electrophoretic mobility shift assay (EMSA). Transient expression assays with NF-kB binding sites linked to the luciferase gene revealed that the increased level of iNOS mRNA and TNF-a mRNA induced by OA were mediated by the NF-kB transcription factor complex. Using DNA fragments containing the NF-kB binding sequence, OA was shown to activate the protein/DNA binding of NF-kB to its cognate site as measured by EMSA. These results demonstrate that OA stimulates NO and TNF-a release and is able to upregulate iNOS and TNF-a expression through NF-kB transactivation, which may be the mechanism, whereby OA elicits its biological effects.

426 ALPHA-HEDERIN AUGMENTS NITRIC OXIDE PRODUCTION AND GENE EXPRESSION IN MURINE MACROPHAGES.

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Alpha-Hederin (HD), a triterpene saponin, is reported to have antitumor activities; however, the mechanism underlying its therapeutic effects is not known. In this study, we examine the effects of HD on the release of nitric oxide (NO) and the

level of inducible nitric oxide synthase (iNOS) gene expression in mouse macrophages. HD elicited a dose-dependent increase in NO secretion. Reverse-transcription polymerase chain reaction showed that the increased NO secretion was due to an increase in iNOS mRNA. Transient expression assays with NF-kB binding sites linked to the luciferase gene revealed that the increased level of iNOS mRNA induced by HD was mediated by the NF-kB transcription factor complex. These results demonstrate that HD stimulates NO release and is able to upregulate iNOS expression through NF-kB transactivation, which may be a mechanism, whereby HD elicits its biological effects.

427 PREVENTION OF ACETAMINOPHEN-INDUCED LIVER TOXICITY BY *PLATYCODON GRANDIFLORUM* IN MICE.

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The protective effects of an aqueous extract from the roots of *Platycodon grandiflorum A.* DC (Campanulaceae), Changkil (CK), on acetaminophen (APAP)-induced hepatotoxicities and the possible protective mechanisms involved were investigated in mice. Pretreatment with CK prior to the administration of APAP significantly prevented the increase in serum alanine aminotransferase and aspartate aminotransferase activity and hepatic lipid peroxidation in a dose-dependent manner. APAP?induced hepatotoxicity was also essentially prevented as evidenced by liver histopathology. Hepatic glutathione levels and glutathione-S-transferase activities were not affected by treatment with CK alone, but pretreatment with CK protected the APAP-induced depletion of hepatic glutathione levels. The effects of CK on cytochrome P450 (P450) 1A2 and 2E1, the major isozymes involved in APAP bioactivation, were investigated. In microsomal incubations, CK effectively inhibited P450 1A2?dependent methoxyresorufin O?deethylase activities and the P450 2E1?dependent p-nitrophenol and aniline hydroxylase. The results suggest that the protective effects of CK against the APAP-induced hepatotoxicity may, at least in part, be due to its ability to block P450?mediated APAP bioactivation. [This work was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea; HMP-00-B-21600-0117]

428 THE HERBAL PRODUCT *SCHIZANDRA CHINENSIS* PROTECTS AGAINST LIPOPOLYSACCHARIDE (LPS)-INDUCED HEPATOTOXICITY IN FISCHER 344 RATS.

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Estimates are that sales of herbal medicines exceeded $4 billion in 1999. A number of herbs are said to have beneficial effects on the liver but scientific evidence of their safety, efficacy and mechanism of action is limited or lacking. *Schizandra chinensis* (Schizandra) has traditionally been used in China for various ailments, from cough to amnesia, and has been touted as a treatment for liver disease. We previously showed that Schizandra protected precision-cut male Fischer F344 rat liver slices against chloroform toxicity *in vitro*. In this *in vivo* study, rats were gavaged daily for 7d with 500µl containing 50, 200, or 500µl of tincture of Schizandra or with 500µl saline (control). The 200µl dose is similar to doses recommended on the bottle for human usage. On day 8, each rat was given 16.66 mg/kg *E. coli* LPS *via* the tail vein along with another dose of Schizandra. After 24h, blood for preparation of serum was collected *via* heart puncture and one lobe of the liver was harvested and fixed in 10% buffered formalin. The other lobe was precision-cut and slices incubated at 37°C for 72h in a Vitron incubator. At 24, 48 and 72h, liver slices were harvested, weighed and assayed for potassium (K^+) and lactate dehydrogenase content as indices of viability. Treatment with Schizandra enhanced maintenance of viability (K^+ content) in liver slices from rats exposed to Schizandra with LPS compared to slices from rats treated with LPS alone. Potassium levels in rats treated with Schizandra and LPS were increased 31% (50 µl dose), 39%, $p<0.05$ (200 µl dose) and 20% (500 µl dose) in liver slices harvested at 48h. There were no significant differences in the weights of the rats in the different groups. These findings demonstrate that Schizandra administered *in vivo*, under the conditions tested, protected liver slices against LPS-induced hepatotoxicity. [Supported by NIEHS Center Grant P30ES06694 (JU), the Richard B. Siegel Foundation (JU, PN) and the UA Undergraduate Biology Research Program (CK, ZL, DC, NY)].

429 INHIBITION OF 2-AMINO-1-METHYL-6-PHENYLIMIDAZO[4, 5-B]PYRIDINE (PHIP)-ASSOCIATED COLORECTAL CARCINOGENESIS BY NATURAL FOOD ANTHOCYANINS IN RATS INITIATED WITH 1, 2-DIMETHYLHYDRAZINE.

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The potential of natural anthocyanin food colors, purple corn color (PCC), purple sweet potato color (PSPC) and red cabbage color (RCC), to modify colorectal carcinogenesis was investigated in male F344/DuCrj rats, initially treated with 1, 2-dimethylhydrazine (DMH) and receiving 2-amino-1-methyl-6-phenylimidazo[4, 5-b]pyridine (PhIP) in the diet. After DMH initiation, PCC, PSPC and RCC were given at a dietary level of 5.0% in combination with 0.02% PhIP until week 36. No anthocyanins-treatment-related changes in clinical signs and body weight were found. Incidences and multiplicities of colorectal adenomas and carcinomas in rats initiated with DMH (25%; 0.3) were clearly increased by PhIP (95%; 2.7). In contrast, lesion development was pronouncedly suppressed by PCC administration (30%; 0.3), and also moderately inhibited by PSPC (70%; 1.4) or RCC (50%; 1.0) treatment. Furthermore, in the non-DMH initiation groups, induction of aberrant crypt foci (ACF) by PhIP was significantly decreased by the RCC supplementation. The results thus demonstrate that while PhIP clearly exerts promoting effects on DMH-induced colorectal carcinogenesis, these can be reduced by PCC, PSPC or RCC in diet, under the present experimental conditions.

430 EFFECT OF DOSE, TIME, AND ROUTE OF ADMINISTRATION OF GENISTEIN ON RADIATION PROTECTION.

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Radioprotective agents have applications in clinical and military settings, space flight, and radiation site cleanup. The estrogenic and antioxidant properties of the soy isoflavone genistein make it an ideal radioprotective candidate. This research investigated the radioprotective efficacy of genistein in CD2F1 male mice. Exp. 1: Mice were each administered a single subcutaneous (sc) dose of genistein either 1 hr or 24 hr before a lethal dose of gamma radiation (9.5-Gy cobalt-60 at 0.6 Gy/min). In both groups, mice either received saline, polyethylene glycol 400 (PEG) vehicle, or genistein at 3, 6, 12, 25, 50, 100, 200, or 400-mg/kg body weight. The 30-day survival rates in the groups treated 1 hr before irradiation were 6%, 13%, 13%, 0%, 0%, 6%, 6%, 6%, 13%, and 19%, respectively. Survival rates in the groups treated 24 hr before irradiation were 8%, 15%, 6%, 0%, 19%, 60%, 56%, 65%, 91%, and 85%, respectively. Thus, genistein did not provide radioprotection at any of the doses when given 1 hr before irradiation, doses of 25-400 mg/kg given 24 hr before 9.5 Gy afforded significant protection. Exp. 2: Mice that each received a single oral gavage of saline, PEG, or 400 mg/kg genistein 1 hr before 9.5-Gy irradiation had 30-day survival rates of 0%, 9%, and 3%, respectively; when given 24 hr before 9.5 Gy, survival rates were 3%, 9%, and 9%, respectively. Thus, a single oral dose of genistein was not radioprotective. Exp. 3: Mice received daily gavages of 100- or 400-mg/kg genistein for either 4 days before, 4 days after, or 4 days before and after irradiation at 9.5 Gy. Survival rates of mice receiving 100-mg/kg genistein were 0%, 0%, and 50%, respectively; for those receiving 400-mg/kg genistein, survival rates were 44%, 0%, and 69%, respectively; and for those receiving saline and PEG, survival rates were 0% and 19%, respectively. This research demonstrates that both a single sc dose and multiple oral doses of genistein protect mice from a lethal dose of gamma radiation.

431 SILYMARIN ATTENUATES THE HEPATOXICITY OF ALPHA-NAPHTHYLISOTHIOCYANATE.

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Silymarin, a polyphenolic flavonoid derived from milk thistle seeds, demonstrates anti-inflammatory and hepato-protective activity against certain toxicants. In rodents, the acute hepatotoxicity of alpha-naphthylisothiocyanate (ANIT) is inflammatory cell-dependent and mirrors that of drug-induced cholestasis in humans. Since silymarin has anti-inflammatory activity and recent studies suggest that it decreases the formation of ANIT-induced hepatic lesions, the hypothesis that silymarin affords protection against ANIT-induced hepatotoxicity was investigated. Male Sprague-Dawley rats were treated for 5 consecutive days with 0-50 mg/kg silymarin (oral). On day 6, the silymarin treated rats received 120 mg/kg ANIT (oral). Parameters of hepatotoxicity were determined 12 and 24 hours after ANIT

treatment. Rat livers were processed for histological analysis of zonal necrosis and plasma samples were collected for analysis of hepato-specific enzyme levels. The histopathological analysis of ANIT-induced liver injury was manifested as pronounced infiltration of neutrophils, overt necrosis to periportal parenchymal cells and injury to bile duct epithelium. In addition, ANIT treatment induces the increased expression of neutrophil associated adhesion molecules, elevated plasma levels of aspartate aminotransferase (AST, SGOT), alanine aminotransferase (ALT, SGPT), bilirubin, and decreased bile flow. The prophylactic administration of 50 mg/kg silymarin afforded hepato-protection against all parameters of ANIT-induced toxicity except bile flow. Importantly, the degree of neutrophil chemotaxis and induction of necrotic lesions was reduced. Moreover, the hepatic expression of adhesion molecules, such as ICAM-1, was decreased significantly. ANIT-induced elevation of plasma levels of ALT, AST, and bilirubin were reduced significantly by silymarin pretreatment. These data suggest that silymarin is protective against the neutrophil-dependent hepatotoxicity of ANIT by, in part, preventing the hepatic-accumulation of circulating neutrophils. (Supported by NIH Grant RR11606-05)

432 DEVELOPMENTAL EXPOSURE OF RATS TO DIETS CONTAINING PACIFIC OCEAN OR LAKE ONTARIO SALMON MODIFY LATER RESPONSES TO LPS.

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Fish contain fatty acids (FA), some of which (*i.e.* n-3) may be beneficial. However, there are large variations in FA content and speciation between fish species as well as in the same species from different waters. Because n-3 FA are anti-inflammatory, we sought to determine whether developmental exposure to diets containing salmon or salmon-extracted oil from either Pacific Ocean (PO) or Lake Ontario (LO) would, in the adult, modify the inflammatory response to LPS. From gestational day 6 through weaning, dams were fed diets containing 20% lyophilized PO or LO salmon, PO or LO oil derived from equivalent amounts of salmon, or control chow. Female offspring, 4 months old, were given LPS (100 µg/kg BW, *i.p.*) and blood was collected for up to 5 h. In control rats, LPS increased serum tumor necrosis factor alpha (TNF_α), interleukin 6 (IL-6), and nitric oxide (NO). However, in offspring of dams fed fish oil from either PO or LO, the TNF_α increase after LPS was diminished by 40%. At 3 h post LPS, rats fed PO oil diet had serum IL-6 lower than the rest. At 5 h, although greater than controls, serum IL-6 in rats fed PO oil was lower than in rats exposed to LO oil. Serum NO at 5 h post LPS was reduced by 70% only in the rats fed PO oil diets. Effects of PO and LO lyophilized salmon diets on serum cytokines and NO after LPS were similar, but less pronounced. FA analysis revealed that PO oil has 5-fold higher n-3/n-6 FA ratio than LO oil. In summary, PO oil is a more potent anti-inflammatory agent than LO oil due, at least in part, to the higher n-3 FA content of PO oil; these effects are long-lasting and are partially masked by other dietary constituents of fish. Thus, dietary FA manipulation early in life may alter sensitivity to inflammatory stimuli in adulthood. (Supported by NIEHS ES079102 and EPA82581201 to RFS; NIEHS ES05879 to NMF).

433 HUMAN PROSTATE CANCER CELLS TREATED WITH SAW PALMETTO EXTRACTS SELECTIVELY ACCUMULATE A BROWN PIGMENTED MATERIAL: CORRELATION WITH ALTERED CELLULAR PROLIFERATION AND MORPHOLOGY.

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To determine the direct effect of commercially available saw palmetto berry extract on the viability and proliferation of human prostate cancer cells in culture, subconfluent cultures of the human prostate cancer cell line, LNCaP, were treated with increasing concentrations of aqueous extracts of saw palmetto oil. Cultures of a transformed human epidermal keratinocyte cell line (HaCaT) were treated in a similar manner for comparison. Following a 24-hour treatment period, the cells were labeled with bromodeoxyuridine and the viability and proliferation of the treated cells were determined using standard flow cytometric methods. Treatment of LNCaP cells with aqueous extracts of saw palmetto oil resulted in a concentration and time-dependent accumulation of a brown pigment within the cytoplasm of the cells. The appearance of this material within the cells was associated with alterations in cellular morphology and a dramatic decrease in the percentage of proliferating cells, without a major effect on the viability of these cells. The development of this pigmented material and its association with the inhibition of proliferation was much less pronounced in the HaCaT cell line, occurring at 10-fold higher concentrations. The results of the current study indicate that the beneficial effects of saw palmetto on pathological conditions of the prostate may be associated with the selective accumulation of this pigmented material in treated prostate cells and its inhibitory effect on cellular proliferation.

434 EFFECTS OF COMBINED QUERCETIN AND COENZYME Q_{10} TREATMENT ON THE OXIDATIVE STRESS PATHWAY IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS.

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Reactive oxygen species may be actively involved in the genesis of a number of pathological states such as ischemia-reperfusion injury, cancer, and diabtes, as well as the aging process. Our objective was to determine if combined dosing of quercetin and coenzyme Q10 (10mg/kg/day ip for 14 days) is beneficial or detrimental in the oxidative stress pathway of 30 day streptozotocin-induced diabetic Sprague-Dawley rats. Blood glucose concentrations were elevated in both normal and diabetic rats after quercetin treatment. Liver, kidney, and heart tissues were excised and the activities of catalase, glutathione reductase, glutathione peroxidase, superoxide dismutase, and concentrations of oxidized and reduced glutathione were determined. In the liver, superoxide dismutase, glutathione peroxidase, and levels of both oxidized and reduced glutathione were significantly decreased from the non-diabetic control, and these effects were not reversed when antioxidants were administered. In kidney, glutathione peroxidase activity was significantly elevated in the diabetic rats as compared to non-diabetic kidney, Antioxidant treatment did not return the enzyme activity to non-diabetic levels. In heart, catalase activity was increased in diabetic animals, and returned to normal by combined treatment with quercetin and coenzyme Q10. Cardiac superoxide dismutase was lower in quercetin and quercetin + coenzyme Q10 treated diabetic rats reversing the effect of diabetes. There were no adverse effects on the oxidative stress pathway after quercetin or coenzyme Q10 singularly or in combination. Quercetin may be effective in reversing some effects of diabetes, although the rise in blood glucose levels may limit the usefulness of therapy.

435 OXIDATIVE STRESS PARTICIPATES IN ACUTE LUNG INJURY AND ACTIVATION OF MITOGEN ACTIVATED PROTEIN KINASES (MAPK) FOLLOWING AIR POLLUTION PARTICLE EXPOSURE (PM).

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Epidemiological reports suggest that PM is associated with increased rates of cardiopulmonary-related morbidity and mortality. *in vitro* studies have demonstrated insights into the mechanism of lung injury caused by PM. To further understand the mechanism of PM injury, we studied the *in vivo* effects of residual oil fly ash (ROFA) exposure on MAPK activation. The MAPK cascade regulates the activity of specific nuclear transcription factors that control genes involved in cell growth, differentiation, apoptosis, and inflammation. A 2×2 factorial arrangement of treatments was used in a randomized design (n=26 male Sprague-Dawley rats, 60-90 d of age). Factors included: 1) pre-treatment with either an intraperitoneal (IP) injection of 500 mg/kg dimethlythiourea (DMTU) or saline, thirty minutes prior to instillation (IT) and 2) IT with 0.5 mg/rat ROFA or saline. Bronchoalveolar lavage fluid was obtained at 24h for determination of cytotoxicity and inflammation. ROFA increased lactate dehydrogenase and induced neutrophilia at 24h post-exposure ($P < 0.05$). IP pre-treatment with DMTU inhibited both the cytotoxicity and neutrophilia ($P < 0.05$). Sixty-three male Sprague-Dawley rats (60-90 d of age) were then randomly assigned as previously described and whole lung samples were collected for analysis at 1h, 3h, and 24h. ROFA increased p-ERK levels at 3h and 24h ($P < 0.05$). These increases were attenuated by DMTU pre-treatment ($P < 0.01$). DMTU inhibited the ROFA induced p-ERK activity increase at 24h ($P < 0.01$). The inhibition of ROFA-induced injury, MAPK expression and MAPK activity by DMTU provides strong evidence that generation of oxidative stress is critical to ROFA-induced cytotoxicity. These data link *in vitro* and *in vivo* results and demonstrate an oxidative mechanism operating through molecular signaling pathways by which environmental factors cause lung injury. (This abstract does not reflect EPA policy.)

436 SUB-CHRONIC EFFECT OF METAL WORKING FLUIDS ON PULMONARY FUNCTIONS IN B6C3F1 MICE: ROLE OF VITAMIN E.

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Metal working fluids (MWFs) are widely used in many industries. Exposures to MWFs are known to cause a spectrum of respiratory effects like excessive cough and attendant secretary products, bronchitis and physical discomfort as evidenced

by tightness of chest. This lead us to examine if an inhalation exposure to a semi-synthetic neat MWF produces any morphological as well as functional alterations in the lungs of mice. Morphological/functional and antioxidant outcomes in lungs were studied after inhalation exposure to MWF of vitamin E deficient and sufficient mice. Mice were given vitamin E deficient or basal diets for 35 weeks. Inhalation exposure to MWF started after 18 weeks on respective diets. Mice were exposed to MWF for 85 individual exposures (17 weeks, five days a week, 6 hours a day). No morphological alterations were seen in the lungs of mice from the studied groups. However, functional deficits in breathing rate, peak inspiratory/expiratory flow, minute ventilation, and tidal volume, along with marked reduction in the lung levels of GSH, protein thiols and ascorbate were in evidence in mice maintained on vitamin E deficient diet. Mice maintained on sufficient diet and exposed to MWF had decreased tidal volume compared to respective controls. Exposure to MWF resulted in accumulation of peroxidative products, and reduction of ascorbate content in the lungs compared to vitamin E sufficient or deficient controls. The results of this study indicate that dietary vitamin E deficiency enhanced subchronic effect of inhalation exposure to MWF.

437 MECHANISMS OF UV INDUCED FREE RADICAL OXIDATIVE DAMAGE IN HUMAN SKIN FIBROBLASTS.

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UV radiation induces the production of oxygen free radicals although there is limited understanding of mechanisms by which UV induces oxidative damage in human skin fibroblasts. We tested the hypothesis that acute exposure to environmental levels of UV radiation in Puerto Rico can cause oxidative damage to fibroblasts leading to apoptosis and necrosis. Environmental UV levels were measured over a period of two and half years and used to establish irradiation doses for human fibroblasts (CRL-2072) using an Oriel 1 KW UV Solar Simulator. Cells were irradiated with three doses: 0.28 (low dose), 0.36 (mean dose) and 0.44 (high dose=5.4 min) J/cm^2 and DNA adducts, DNA repair capacity, lipid peroxidation, apoptosis and necrosis measured. A low dose did not caused changes in guanosine and 2-deoxyguanosine (2dG). However, the mean dose resulted in increases of 407% and 43% in guanosine and 2-dG respectively. The high dose caused an increase of 331% and 34% in guanosine and 2dG, respectively and a 240% increase in lipid peroxidation. The DNA repair capacity of these cells increased after acute exposure to low and mean doses but decreased with a high UVA/UVB dose. Acute exposure to the equivalent of 1.5 hours of environmental levels of UV caused necrosis in all fibroblast cells. These results show that even a short-term, acute exposure to environmental levels of UV can cause substantial oxidative damage resulting in apoptosis and necrosis. Supported by NASA grant NAG-2-1176 to J.L.M.

438 N-ACETYLCYSTEINE PRETREATMENT DECREASES COCAINE AND ENDOTOXIN INDUCED HEPATOTOXICITY.

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Cocaine (COC) produces hepatotoxicity by a mechanism, which remains undefined, but has been linked to its oxidative metabolism. Endotoxin (lipopolysaccharide, LPS) is also a well-known cause of hepatic damage, due to glutathione (GSH) depletion and oxidative stress. Previously we have demonstrated that exposure to non-injurious doses of LPS dramatically increases cocaine mediated hepatotoxicity (CMH). N-Acetylcysteine (NAC) protects against oxidative stress and depletion of GSH. This study was conducted to investigate if NAC pretreatment inhibits LPS potentiation of CMH. For five consecutive days, male CF-1 mice were administered oral NAC (400 mg/kg) or sterile saline, followed an hour later with COC (20 mg/kg) or sterile saline. On the last day, four hours after the last COC or saline treatment, the mice were administered 12 x 10^6 EU LPS/kg or sterile saline. The mice were sacrificed 18 hours later by decapitation. For the COC alone and COC + LPS groups, NAC pretreatment, significantly decreased serum ALT and AST activities, indicating reduced liver injury. In addition, the blood GSH content in the COC alone and COC + LPS groups pretreated with NAC was 25% higher *versus* the mice pretreated with saline. Moreover, in all groups pretreated with NAC, hepatic GSH concentration significantly increased as well as the hepatic and blood glutathione peroxidase (GPx) and catalase (CAT) activities. In conclusion, the results demonstrate that NAC pretreatment exerted a protective effect against LPS potentiation of CMH.

439 AMELIORATION BY PHYSICAL CONDITIONING OF CARDIAC OXIDATIVE INJURY CAUSED BY CHRONIC NITRIC OXIDE SYNTHASE INHIBITION IN RAT.

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Many individuals with cardiac diseases undergo periodic physical conditioning with or with out medication. Therefore, this study investigated the interaction of physical training and chronic nitric oxide synthase (NOS) inhibitor (Nitro-L-Arginine Methyl Ester, L-NAME) treatment on blood pressure (BP), heart rate (HR) and cardiac oxidants/antioxidants in rats. Fisher 344 rats were divided into four groups and treated as follows: 1) sedentary control, 2) Exercise training for 8 weeks, 3) L-NAME (10 mg/kg, s.c. for 8 weeks) and 4) ET + L-NAME. The animals were sacrificed 24 hr after last treatments and hearts were isolated and analyzed. Physical conditioning resulted in a significant increase in respiratory exchange ratio (RER), cardiac NO production, NOS activity and endothelial eNOS and inducible iNOS protein expression. Training significantly enhanced cardiac GSH levels, GSH/GSSG ratio and up-regulation of cardiac CuZn-SOD, Mn-SOD, catalase (CAT) glutathione peroxidase (GSH-Px) activity and protein expression. Training also caused depletion of cardiac malondialdehyde (MDA) level, protein carbonyl content. Chronic L-NAME administration resulted in depletion of cardiac NO level, NOS activity, eNOS, nNOS and iNOS protein expression, GSH level, GSH/GSSG ratio and down-regulation of cardiac CuZn-SOD, Mn-SOD, CAT, GSH-PX activity and protein expression. Cardiac xanthine oxidase (XO) activity increased with enhanced MDA level and protein oxidation after L-NAME administration. The biochemical changes were accompanied by increased in BP and HR. Interaction of training and chronic NOS inhibitor treatment resulted in normalization of BP, HR and up-regulation of cardiac antioxidant defense system.It is concluded that physical conditioning attenuated the oxidative injury caused by chronic NOS inhibition by up-regulating the cardiac antioxidant defense system and lowering the BP and HR in rats(supported by AHA/IA grant # 0051395Z).

440 α-TOCOPHEROL OR INDOMETHACIN PRETREATMENT PREVENTS SEIZURE-INDUCED INCREASES IN F_2-ISOPROSTANES AND CITRULLINE.

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Free radicals, particularly the reactive oxygen or nitrogen intermediates, are thought to be involved in kainic acid (KA) toxicity, exacerbating excitotoxicity and exerting tissue damage. F_2-isoprostanes (IsoP) and F_4-neuroprostanes (NP) are highly accurate biomarkers of oxidant injury and are products of free radical-catalyzed peroxidation of arachidonic acid and docosahexaenoic acid, respectively. Activation of nitric oxide synthase (NOS), resulting in an increase of nitric oxide (NO) and citrulline, has also been suggested to be part of KA toxicity. As a measure of oxidative stress, lipid peroxidation and NOS activity, changes in IsoP, NP and citrulline levels were studied in the brain of C57Bl/6 mice exposed to intracerebroventricular administration of KA (1 nmol/5 µl). KA produced severe seizures in mice immediately after administration and a significant increase in IsoP (158%), NP (237%) and citrulline (249%) levels were seen 30 min following treatment compared to controls (100%). No significant increase in IsoP and citrulline levels were found in mice pretreated with α-tocopherol (100 mg/kg, i.p.) for 3 days or those exposed to indomethacin (inhibitor of cyclooxygenase, COX, 14 µg/ml of drinking water) for 2 weeks prior to KA treatment. These findings provide novel information about KA-induced excitotoxicity and the relationship of seizures to free radicals, COX and NO. (Supported by AG 05144, AG 16835 and ES04597).

441 IMMUNOCHEMICAL DETECTION AND LOCALIZATION OF MALONDIALDEHYDE-PROTEIN ADDUCTS IN THE SPLEENS OF ANILINE-TREATED RATS.

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We have reported previously that aniline exposure in rats causes increased lipid peroxidation and formation of malondialdehyde (MDA)-protein adducts in the spleen. In order to further elucidate the role of MDA-protein adducts in the splenic toxicity of aniline, studies were conducted to detect and localize these adducts in the spleen. Rabbit polyclonal antisera to MDA-KLH were employed for Western blot analyses and immunohistochemical localization of MDA-protein addcuts in the spleens of aniline-treated (65 mg/kg/day aniline in the drinking water for 30 days) and control rats. Western blot analyses of splenic microsomal proteins from aniline-treated and control rats showed the presence of 13 MDA-modified proteins. However, 26, 32 and 44 kDa proteins were more prominent in the aniline-

treated rats. For immunohistochemical localization of MDA-protein adducts in the spleen, a new approach using alkaline phospharase-Fast red (red color) demonstration of bound primary antibodies was adopted, providing a sharper and increased contrast compared to horseradish peroxidase-diaminobenzidine (brown color) methodology. This new approach allowed us to differentiate the changes in aniline-treated spleens, which had extensive brownish deposits of iron. Spleens from aniline-treated rats showed intense staining for these adducts in the red pulp areas (where iron was also localized), especially within the sinusoidal macrophages. Spleens from control rats showed only mild staining. The co-localization of MDA-protein adducts with iron in the red pulp of the spleen provides a potential mechanism of splenic toxicity of aniline.

442 BENZO(A)PYRENE-QUINONES (BPQS) GENERATE REACTIVE OXYGEN SPECIES (ROS) AND ARE MITOGENIC IN LYMPHOID AND NON-LYMPHOID CELLS.

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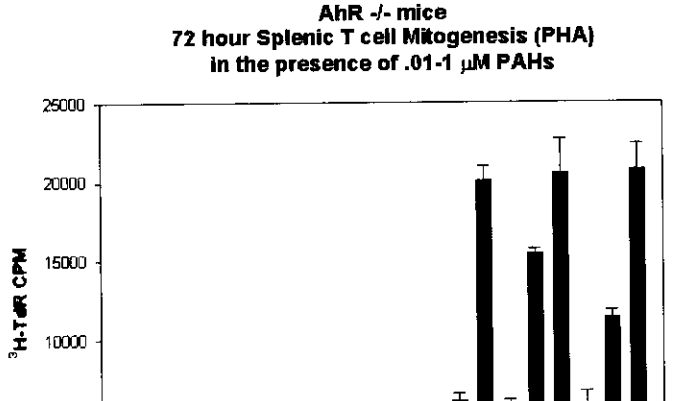
Previous studies have shown that quinones (diones) of benzo(a)pyrene (BaP) are formed in the presence of ultraviolet light and under the influence of various P450s and peroxidases. We have shown that BPQs are mitogenic in human lung epithelial cells (Reed et al, submitted) and human mammary epithelial cells (Burdick et al, submitted). We have also shown that BPQs generate reactive oxygen species (ROS) as detected using fluorescent ROS-reactive substrates including DCHF and DHE. In this presentation, we will compare the effects of BPQs on lung and mammary epithelial cells. In addition, we present new data indicating that BPQs are highly mitogenic in murine spleen cells, including those obtained from AhR -/- null mice. In these studies, AhR -/- spleen cells treated with 0.1-1 μM 3, 6-BPQ, 1, 6-BPQ, and 6, 12-BPQ had nearly a tenfold increase in cell proliferation in a 3-day T cell mitogen (phytohemagglutinin) culture. BaP (0.1-1 μM) was not immunosuppressive to T cell mitogenesis in AhR -/- spleen cells. The results of these studies demonstrate that mild oxidative stress in various lymphoid and nonlymphoid cells is mitogenic, perhaps owing to activation of the epidermal growth factor receptor (EGFR) in epithelial cells or other mitogenic receptors in lymphoid cells. This work was supported in part by NIH grant ES-05495, ES-07259, ES-10433, and P20 ES-09871



443 ROLE OF NITRIC OXIDE (NO) IN THE DEPLETION OF HIGH-ENERGY PHOSPHATES IN SKELETAL MUSCLES OF RATS ACUTELY EXPOSED TO DFP.

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The objective of this investigation was to determine the acute effects of the AChE inhibiting organophosphate compound DFP (1.5 mg/kg, s.c.) on citrulline (determinant of NO) and energy-rich phosphates (ATP and phosphocreatine) and their major metabolites in fast and slow skeletal muscles. Citrulline and energy metabolites were determined in perchloric acid extracts of muscles frozen *in situ* using HPLC methods. Analyses of controls revealed significantly higher levels of citrulline in the soleus (451.2±5.3 nmol/g), than in the EDL (381.3±8.2 nmol/g) or diaphragm (331.2±9.9 nmol/g). In contrast, the levels of ATP and phosphocreatine (PCr) were higher in EDL than in the soleus or diaphragm. Within 15 min of DFP exposure, with onset of fasciculations, citrulline levels were significantly elevated in all three muscles. Maximum increase in citrulline (272-288%) was noted after 60 min. At this time point, the levels of ATP, total adenine nucleotides (TAN), PCr, and total creatine compounds (TCC) were maximally reduced in all three muscles. The findings revealed that the increase in ROS (measured as increased NO synthesis) preceded the decrease of ATP in skeletal muscles. NO is known to exert neurotoxic effects primarily by depletion of cellular energy stores through multiple mechanisms, including inhibition of mitochondrial respiratory enzymes, particularly cytochrome-c oxidase, at a time when a greater utilization of ATP is necessary during muscle hyperactivity. Thus, it can be concluded that increased ROS generation may be the first step in the mechanisms involved in DFP-induced loss of energy metabolites, in addition to greater utilization of ATP, causing mitochondrial dysfunction and muscle neurotoxicity. (Supported by NIH grant, ES04597).

444 ACRYLONITRILE POTENTIATES NOISE-INDUCED HEARING LOSS: A CONSEQUENCE OF OXIDATIVE STRESS?

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Previous research from this laboratory (Rao, 2000; 2001) has shown that promotion of oxidative stress by asphyxiants + noise results in cochlea injury and auditory impairment. In this study we sought to determine whether acrylonitrile, an agent that significantly depletes glutathione would also promote noise-induced hearing loss. Acrylonitrile is commonly used in the production of plastics and synthetic fibers. It also ranks among the top 50 high volume chemicals produced in the US alone. Although acrylonitrile is a suspected human carcinogen, the neurotoxic effects are not clearly understood. In this study we determined the effects of acrylonitrile on auditory function and also on cochlear glutathione levels. Long Evans hooded rats were exposed to acrylonitrile, noise plus acrylonitrile, noise alone, and no experimental treatment (controls). Following a recovery period of three weeks, auditory thresholds were tested electrophysiologically. Results demonstrate that the auditory thresholds from animals administered acrylonitrile and exposed to noise had much higher thresholds than animals exposed to noise alone. Acrylonitrile produced no adverse effects on auditory function by itself. Spectrophotometric assay of glutathione levels in the cochlea following acrylonitrile administration demonstrates a marked reduction of glutathione compared to controls. We hypothesize that acrylonitrile potentiates noise-induced hearing loss by rendering the cochlea more sensitive to oxidative stress. Supported in part by ES08082.

445 OXIDATIVE STRESS INDUCED IN MURINE SPLENOCYTES AFTER EXPOSED TO MULTIPLE PESTICIDES *IN VITRO*.

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The widespread use of pesticides in the environment increases the likelihood of multiple pesticide exposure, either concurrently or sequentially, over an organism's lifetime. This study examines both pro-oxidant and antioxidant status in murine (C57BL/6) splenocytes exposed to individual pesticides and pesticide mixtures, *in vitro*. The three insecticides of interest are lindane (Lin; an organochlorine), malathion (Mal; an organophosphate) and piperonyl butoxide (PBO; a synergist). These three pesticides were found to be cytotoxic to splenocytes in both concentration- and time- dependent manner. Based on minimum cytotoxicity ($\leq LC_{25}$), the following concentrations had been chosen for this study: 70μM Lind, 50μM Mal and 55μM PBO. The pro-oxidant and antioxidant statuses of the cells were monitored as indices of oxidative stress. The pro-oxidant hydrogen peroxide (H_2O_2) activity was monitored *via* the dichlorofluorescin diacetate assay. Exposure to pesticides for 15 minutes significantly increased H_2O_2 production above the controls: Mal 21.1%; Lind 10.8%; PBO 25.9%; Mal+Lind 26.8%; Mal + PBO 37.8%. The activities of superoxide dismutase and catalase levels in splenocytes were not significantly altered by these pesticides and their mixtures when exposed for 12 hrs. However, glutathione reductase (GSH-Rx) and glutathione peroxidase (GSH-Px) levels were significantly reduced when exposed to pesticide mixtures. Thus, GSH-Rx levels were found to be: control 51.7; Mal 48.2; Lind 50; PBO 52.3; Mal + Lind 40.5; Mal + PBO 42 Units/mg. The GSH-Px activity levels were found to be: control 44.9; Mal 30.2; Lind 30.6; PBO 32.4; Mal + Lind 21.1; Mal + PBO 21.1 Units/mg. These results indicate that exposure to mixtures of Mal, Lind and PBO

induces immunotoxicity, at least in part, *via* oxidative stress by both increased pro-oxidants and decreased antioxidants in cells in an additive manner compared to the individual pesticide exposures.

446 THE OXIDATIVE STRESS LEVELS OF WORKERS WHO HAVE POSSIBLE EXPOSURE TO MUNICIPAL INCINERATOR FLY ASH.

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It is well known that municipal solid waste incinerator (MSWI) fly ash includes heavy metals, dioxins, polyaromatic hydrocarbons (PAH), and other organic materials. According to *in vitro* and animal studies, heavy metals and dioxins probably cause oxidative stress and PAH may also cause oxidative stress during metabolism in human body. In this study, we measured the levels of several oxidative stress markers in the blood and urine specimens of MSWIs workers and discussed whether the duration of exposure to MSWI fly ash was associated with the levels of oxidative stress markers. The subjects were 81 male workers (mean age 42.7) from four MSWIs in the same city. Written informed consents were given from all subjects. Occupational health doctors interviewed each subject about his job history, evaluated the exposure to fly ash, and calculated its total duration. The subjects were classified into four groups; those were Long duration of exposure to fly ash, Short duration of exposure to fly ash, Limited exposure to fly ash, and Control. Blood and urine specimens were obtained from subjects in the morning. The levels of 8-hydroxydeoxyguanosine (8-OHdG) in urine and in leukocytes were measured as oxidative DNA damage makers. Blood malondialdehyde (MDA) and lipid peroxides (LPO) levels, and total urinary biopyrrins level were also measured as oxidative stress markers. α-Tocopherol was measured as major antioxidant in serum. The mean levels of all markers were compared among four groups. Although the levels of all markers had no significant differences among groups, the tendency that the level of urinary 8-OHdG elevated with the increased duration of exposure to MSWI fly ash was significant. Considering this result, some chemicals contained in MSWI fly ash might have induced oxidative stress in the workers.

447 MODULATION OF PMN FUNCTION BY INCUBATION WITH HUMAN PLASMA FROM OXIDANT-CHALLENGED INDIVIDUALS.

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Polymorphonuclear leukocytes (PMN) play a fundamental role in the primary host defense against bacterial pathogens. It is possible that PMN function might be modulated by soluble immune mediators released into the blood stream due to oxidative stress. PMN from healthy volunteers were tested for the stimulated release of superoxide anions after being incubated with plasma of: 1. Welders and a matched (age and smoking habits) non-welders group 2. Smokers and non-smokers 3. Elderly and young individuals. The experiments showed that stimulated superoxide production was inhibited ($p<0.05$) by plasma from smokers as compared to that of non-smokers and by plasma from elderly individuals as compared to that of younger ones. There was no statistical difference between superoxide release from PMN incubated with plasma from welders as compared with that from an age and smoking habits non-welders matched group. These findings indicate that age and smoking induce the appearance in plasma of factors which affect neutrophil function. Determination of these factors could elucidate the mechanism of impaired immune response in elderly and oxidant-challenged individuals.

448 INDUCTION OF METALLOTHIONEIN SYNTHESIS BY MITOCHONDRIAL OXIDATIVE STRESS.

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Mitochondria is a major source of reactive oxygen species (ROS). The harmful effects of mitochondrial ROS have been implicated in the aging process and neurodegenerative diseases, but antioxidant systems against mitochondrial ROS are not fully understood. Metallothionein (MT), a cysteine-rich and metal-binding protein, is an efficient scavenger of oxygen radicals, such as superoxide and hydroxyl radicals, but the induction of MT by mitochondrial ROS have never been investigated. In this study, we examined the induction of MT synthesis by administration of mitochondrial-specific ROS generators such as antimycin A (AA), an electron transfer inhibitor, and 2, 4-dinitrophenol (DNP), an uncoupling agent. Subcutaneous administration of AA or DNP to mice significantly increased the hepatic MT concentration in a dose- and time-dependent manner. Administration of AA or DNP increased hepatic lipid peroxide production, and AA elevated mitochondrial lipid peroxide production in the liver. DNP or AA treatment elevated the hepatic lipid peroxidation and serum glutamine-pyruvate transaminase (GPT) levels. The inhibition of glutathione (GSH)Å@synthesis by L-buthionine-SR-sulfoximine (BSO) enhanced the liver injury caused by DNP or AA administration. Moreover hepatic MT concentrations of DNP- or AA-treated mice were increased by BSO-pretreatment. The intracellular accumulation of hydroperoxide by AA or DNP treatment in MT-/- fibroblast cells was higher than that in MT+/+ cells, suggesting that MT plays a role in regulation of intracellular reactive oxygen levels caused by mitochondrial stress. Taken together, these results suggest that mitochondrial oxidative stress induces MT synthesis, which may contribute to regulation of production of mitochondrial reactive oxygen.

449 GENERATION OF REACTIVE OXYGEN SPECIES IN BREAST-CANCER CELL CULTURES AFTER TREATMENT WITH CLARIFIED SLURRY OIL AND CRUDE OILS.

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Clarified slurry oil (CSO), and two crude oil samples, Belridge Heavy Crude Oil (BHCO) and Lost Hills Light Crude Oil (LHLCO) were examined for their ability to generate reactive oxygen species (ROS) in human breast-cancer (MCF-7) cell cultures. Oil samples were prepared in DMSO resulting in extraction of virtually all of the aromatic hydrocarbons including the 3-7 ring polycyclic aromatic compounds (PACs) comprising 62.2% of the CSO, 9.0% of the BHCO, and 2.0% of the LHLCO by total weight. Generation of ROS was measured in a Coulter Epics flow cytometer using 20 μM dihydrorhodamine 123 (DHR), and cell viability was determined using 5 μM propidium iodide (PI). In the acute treatment experiments, single-cell suspensions were loaded with DHR and PI and then treated with the oil samples (1 ppm or 10 ppm). Measurements were made at 5, 15, 30, 60 and 90 minutes after addition of oil samples. In the chronic experiments, pre-confluent cell cultures were treated with oil samples for 6, 12, or 24 hours prior to preparing single-cell suspensions that were loaded with DHR and PI. Acute treatment with CSO, BHCO or LHLCO resulted in no increase in the generation of ROS as compared to the positive control, hydrogen peroxide. Chronic treatment with the CSO sample resulted in a significant increase in ROS at 24 hours. Treatment of cell cultures with a single polycyclic aromatic hydrocarbon, BENZO(A)PYRENE, resulted in a significant and concentration-dependent generation of ROS at 24 hours. (Supported by NIEHS Award RO1 ES049795-01A1 and TUBITAK-NATO).

450 OXIDATIVE DAMAGE TO RAT ERYTHROCYTE MEMBRANE LIPIDS AND SKELETAL PROTEINS BY MAQ-NOH, AN N-HYDROXY METABOLITE OF THE ANTIMALARIAL DRUG, PRIMAQUINE.

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We have shown previously that 6-methoxy-8-hydroxylaminoquinoline (MAQ-NOH), a potential human metabolite of the antimalarial drug, primaquine, is a direct-acting methemoglobinemic and hemolytic agent in rats. To examine the mechanism underlying the hemolytic activity, the present studies were undertaken to determine the effect of MAQ-NOH on rat erythrocyte sulfhydryl status, membrane lipids and skeletal proteins, and morphology under conditions known to commit the cells to premature removal from the circulation. Treatment of rat erythrocytes with MAQ-NOH (350-750 μM) depleted GSH with concomitant formation of glutathione-protein mixed disulfides. MAQ-NOH also induced a concentration-dependent formation of thiobarbituric acid-reactive substances and F2-isoprostanes, indicating the occurrence of lipid peroxidation. SDS-PAGE and immunoblot analysis of erythrocyte ghosts using an anti-hemoglobin antibody revealed that MAQ-NOH treatment had no effect on membrane skeletal proteins, despite a concentration-dependent increase in the content of membrane-bound hemoglobin monomer. Furthermore, scanning electron microscopy revealed that MAQ-NOH had no effect on red cell morphology. The data are consistent with the concept that MAQ-NOH induces erythrocyte membrane damage by an oxidative stress, however, the mechanism underlying its hemolytic activity may differ from that of other hemolytic N-hydroxylamines, such as dapsone hydroxylamine. Supported by NIH grant AI46424.

451 FREE RADICAL PRODUCTION AND OXIDATIVE STRESS IN CYCLOOXYGENASE-2 TRANSFECTED PC-12 CELLS.

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Expression of cyclooxygenase-2 (COX-2) and production of prostaglandins are elevated after ischemia, while inhibition of COX-2 increases viability of neuronal cells. COX-2 has been reported to produce free radicals (e.g., arachidonic acid free radical (AA*;)), during its catalytic cycle and induce oxidative stress. Therefore, COX-2 may be a major contributor to oxidative stress during ischemia/reoxygenation. We have tested whether oxidative stress is enhanced in COX-2-transfected PC-12 cells. By spin trapping techniques, we observed and identified COX-2-dependent AA*; production. For COX-2 delivery, we incubated CHARIOT™; with recombinant COX-2 protein to yield a non-covalent complex, which was subsequently added to cells for internalization. Using Western blotting, we found that COX-2 protein amounts in PC-12 cells were significantly increased. The COX-2 activity was increased around 30-fold in transfected cells, as determined by a STAT-prostaglandin E2 EIA kit. We used the sensitive HPLC fluorescent technique based on metabolic integration of oxidation-sensitive fluorescent fatty acid, parinaric acid, into membrane phospholipids of cells to measure phospholipid peroxidation. After exposure of transfected PC12 cells to AA (50 µM, 15 min) only one phospholipid, phosphatidylserine, was preferentially oxidized. The AA-induced peroxidation could be partly blocked by a COX-2 inhibitor, NS-398, indicating that peroxidation is COX-2 dependent. To detect AA•, we used the spin trap -(4-pyridyl-1-oxide)-N-t-butylnitrone (POBN). Interaction of COX-2 and AA in the presence of POBN gives rise to an EPR-detectable pentyl radical spin adduct (with hyperfine splitting constants: aN = 15.80 Gs, aH = 2.66 Gs). This EPR signal was suppressed by a COX-2 inhibitor, nimesulide. Our observations demonstrate that COX-2 generated pentyl radicals may be responsible for oxidative stress associated with catalytic activity of the enzyme.

452 4-HYDROXYNONENAL METABOLISM IN ADULT AND PRENATAL LIVER SUBCELLULAR FRACTIONS.

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A number of highly reactive unsaturated aldehydes are produced during cellular oxidative injury. Of these compounds, 4-hydroxynonenal (4HNE) is particularly abundant and highly cytotoxic due to its long half-life and its ability to form stable adducts with proteins and nucleic acids. In the current study, we examined the comparative metabolism of 4HNE by aldehyde-metabolizing enzymes in adult and second trimester prenatal liver subcellular fractions. The predominant biochemical pathways for 4HNE detoxification include alcohol dehydrogenase (ADH), aldehyde reductase (ALRD), aldehyde dehydrogenase (ALDH) and glutathione S-transferase (GST). The initial rates of cytosolic ALDH-catalyzed 4HNE oxidation, ALRD-catalyzed 4HNE reduction, and ADH-catalyzed 4HNE reduction were 8-, 2-, and 40-fold higher, respectively, in adult liver than in prenatal liver. Similarly, mitochondrial ALDH activities were 7-fold higher in adult liver than in prenatal liver. In contrast, affinity-purified cytosolic GST from adult and prenatal liver catalyzed the conjugation of 4HNE with glutathione at relatively similar rates. Our results suggest that second trimester prenatal liver has an overall lower ability to enzymatically detoxify 4HNE relative to adults. Furthermore, GST conjugation may be a particularly important pathway in protecting the fetus from transplacental prooxidant drugs or chemicals that generate 4HNE. Supported by NIH ES-09427.

453 RAPID ENRICHMENT OF INNER MITOCHODRIAL MEMBRANE WITH ALPHA-TOCOPHEROL FOLLOWING ACUTE VITAMIN E SUCCINATE ADMINISTRATION PROTECTS HEPATOCYTES FROM OXIDATIVE DAMAGE.

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Drug-induced liver injury has become the leading cause of acute liver failure resulting in the need for liver transplantation and in the withdraw of drugs from clinical use. Since scientific evidence supports mitochondria (MITO) as a critical site for the induction of oxidative stress and cell death in acute drug-induced hepatotoxicity, an attractive protection strategy would be to enrich hepatic mitochondria with the antioxidant, d-α;-tocopherol (T). Accordingly, we report that a single ip dose of d-α;-tocopheryl succinate (TS) can rapidly enrich MITO and inner mitochondria membranes (IMM) of rat liver with protective levels of T in contrast to acute T or tocopheryl acetate (TA) administration. The enrichment of MITO with T (*via* TS administration) eliminated MITO membrane susceptibility to lipid peroxidation and protected against Fe^{2+}-induced MITO permeability transition. In addition, hepatocytes isolated from TS pretreated rats were resistant to toxic oxidative damage caused by MITO toxicants such as rotenone and thenoyltrifluoroacetone, mitochondrial complex I and II inhibitors respectively, in combination with the glutathione inhibitor, diethyl meleate. In contrast, acute T administration, at an equimolar concentration enriched liver homogenates but not MITO or IMM with T and did not protect against MITO toxicant-induced oxidative hepatocyte death, indicating that mitochondria are a critical site for oxidative damage leading to acute cell death. We suggest that TS acts as a unique delivery system for T, rapidly accumulating in MITO and IMM to prevent mitochondrial damage and cell death. We propose that the acute administration of TS may prove useful for the therapeutic treatment or prevention of oxidative stress-mediated acute liver injury. Supported by NIH grant #R01ES05452 and Gasper and Irene Lazzara Foundation

454 CYCLOOXYGENASE-2 CONTRIBUTES TO OXIDATIVE STRESS INDUCED BY TEMPORARY FOCAL BRAIN ISCHEMIA.

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Focal cerebral ischemia is associated with a local inflammatory response that participates in the progression of ischemic brain injury. Cyclooxygenase-2 (COX-2) exacerbates ischemic injury of the brain. COX-2 catalyzes the formation of prostaglandins from arachidonic acid (AA) *via* cyclooxygenase/peroxidase cycle. Peroxidase reactions can generate radicals independent of AA metabolism. Thus COX-2 may contribute to oxidative stress during temporary focal brain ischemia. We found that total antioxidant reserves of rat brain as well concentrations of ascorbate and GSH were decreased after 6 and 24h of temporary focal brain ischemia in rats. A selective COX-2 inhibitor, SC58125, prevented depletion of ascorbate and GSH in ischemic rat brain. Next, we used COX-2 null and wild type mice to investigate the role of COX-2 in the mechanism of cerebral injury. We found that MCA occlusion induced a significant decrease of ascorbate and GSH in COX (+/+) but not in COX-2 (-/-) mice. In COX (+/+) mice, ischemia produced higher steady-state concentrations of ascorbate radicals in brain homogenates then in COX (-/-) mice or controls. Addition of AA to brain homogenates resulted in additional increase of ascorbate radical EPR signal. In line with this, the ascorbate radical EPR signal was significantly lower in brain homogenates of rats pretreated with SC58125 before ischemia (SC58125 did not affect ascorbate radical in the presence of a non-specific redox catalyst, Fe2+). Overall, our data suggest that COX-2 contributes to the generation of free radicals and oxidative stress after temporary focal brain ischemia.

455 PEROXYNITRITE FORMATION PRECEDES CELL DEATH IN MIDZONAL NECROSIS DURING LOW FLOW HYPOXIA TO PERFUSED RAT LIVERS.

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Low flow perfusion of isolated rat livers results in early hypoxic hepatocellular necrosis at the border between normoxic periportal and anoxic pericentral hepatocytes. This midzonal region is postulated to have increased superoxide production. Nitric oxide and superoxide undergo a diffusion-limited reaction to form peroxynitrite, a potent oxidant that has been shown to be produced in ischemic heart immediately after reperfusion. Our aim was to assess peroxynitrite formation in perfused rat livers during low flow hypoxia. Rat livers were perfused *via* the portal vein with Krebs-Heinseleit bicarbonate (KHB) buffer saturated with 95% O_2/5% CO_2 at 3-4 ml/min/g of liver. After 20 min of normoxic perfusion, flow rates were decreased to 0.6 ml/min/g for varying times (30, 60 and 120 min) . Subsequently, the livers were infused with KHB containing 0.5 mM trypan blue for 10 min and then with 2% paraformaldehyde in KHB for fixation. To detect peroxynitrite generation, nitrotyrosine adducts were identified by immunohistochemistry using rabbit polyclonal anti-nitrotyrosine antibody and a peroxidase detection system. After 30 min of hypoxia, an increase in hepatocellular nitrotyrosine labeling was observed in midzonal regions. Nitrotyrosine labeling increased further at one hour and was observed in both viable and non-viable hepatocytes, as assessed by trypan blue uptake. After two hours, nitrotyrosine labeling and necrosis expanded from midzonal regions into pericentral regions. Since nitrotyrosine labeling occurred in both viable and non-viable hepatocytes, we conclude that peroxynitrite formation began prior to cell death. We also conclude that both nitric oxide and superoxide are generated at the border between anoxic and normoxic tissue, which leads to peroxynitrite formation. This peroxynitrite generation may contribute to early midzonal necrosis during low flow hypoxia in the liver.

456 DELAYED INTERACTIVE EFFECTS OF SARIN, PYRIDOSTGMINE AND EXERCISE ON THE HEPATIC ANTIOXIDANT DEFENSE SYSTEM IN MICE.

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Pyridostigmine (PYR), a reversible cholinesterase inhibitor, has been employed as a pretreatment drug for possible nerve agent exposure during the Gulf war. The Gulf war veterans were exposed to risk factors such as pesticides, medical agents and possible low levels of nerve gas. Physical stress may increase hepatic ROS production, leading to oxidative tissue damage. It is hypothesized that under stressful conditions during war, PYR and/or sarin could affect the antioxidant system in liver. Hence, this study investigated the interactive effects of PYR, low-dose sarin and exercise training on hepatic antioxidant defense system in mice. Male NIH Swiss mice were divided into eight groups and treated as follows: (1) Control; (2) Sarin [0.01 mg /kg, s.c.; 1/20 LD50]; (3) Exercise training; (4) Sarin plus exercise; (5) PYR [1.2 mg/kg, p.o.]; (6) PYR plus exercise; (7) PYR plus Sarin; (8) PYR plus Sarin plus exercise. Exercise was given for 10 weeks, however PYR and sarin were administered daily during 5th and 6th weeks. The animals were sacrificed 24 hours after the last treatments and livers isolated. SOD activity significantly decreased in groups 3 and 6 compared to control. Catalase activity were reduced significantly to 70%, 53% and 71% of control in groups 3, 5, and 6 respectively. GSHPx activity significantly reduced (70% of control) in Group 6. GR activity decreased in Groups 3 and 6 compared to control. Malondialdehyde significantly increased 124% and 173% of control in Groups 3 and 5 respectively. This study suggest that exercise (physical stress) plus PYR lead to significant alteration in antioxidant enzyme activity and lipid peroxidation, which may produce oxidative injury in hepatic tissue of mice. (In part supported by US Army contract # DMAD 17-97-C-70 and SIU School of Medicine)

457 FORMATION OF OXYGEN RADICALS AND RELEASE OF NITRIC OXIDE (NO) IN RATS UPON EXPOSURE TO HIGH LEVELS OF BLAST OVERPRESSURE: IS NO A MODERATOR OF CIRCULATORY OXIDATIVE STRESS?

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Exposure to blast overpressure (BOP) has been previously shown to result in lung injury characterized by alveolar rupture and pulmonary hemorrhage leading to oxygen insufficiency and oxidative stress (OxS) to the circulatory and cardiopulmonary systems. These events may induce a compensatory release of endogenous nitric oxide (NO), a vasodilator and antioxidant that, in turn, would decrease the extent of OxS *via* redox interaction between NO and circulatory oxygen radicals (OxR). To further examine this hypothesis, deeply anesthetized Sprague-Dawley rats were exposed to BOP shock waves of magnitude 100±10 kPa. After exposure, blood, lung, heart, liver, and spleen samples were analyzed for OxR formation using low-temperature EPR spectroscopy. Three distinct oxygen species: peroxyl radicals (PxR) (g = 2.027), alkoxyl radicals (AxR) (g = 2.015), and low-spin ferric-peroxide complexes (FerProx) (g = 1.978), as well as semidehydroascorbyl radicals (AscR) (g = 2.005, A_H = 2 G), were observed in blood and perfused lung at 10 min following BOP exposure. Release of NO was revealed by formation of nitrosyl hemoglobin in blood (with splitting constant for ^{14}N A_z = 17.2 G), and trapping of NO with iron-(dithiocarboxy)sarcosine complexes (DTCS2-Fe) in lung (adducts with $g_\perp$ = 2.042 and $g_\parallel$ = 2.020) at 20 min following BOP exposure. The release of endogenous NO was accompanied by disappearance of EPR signals of circulatory OxR species (AxR, PxR, and FerProx) and attenuation of the EPR signal of AscR. These observations suggest that endogenously released NO may moderate BOP-induced oxidative stress *via* interaction with circulatory OxR.

458 TOXICITY AND OXIDATIVE INJURY IN PC-12 CELLS TREATED WITH MN(II) AND MN(III).

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Manganese (Mn) is an essential element, but excessive exposure causes neurotoxicity with symptoms similar to Parkinson's Disease. Despite much research, the mechanism of manganese neurotoxicity is still unknown. An area that has received relatively little attention is the impact of manganese speciation on toxicity. Manganese does not exist as a single species *in vivo*, but is found as ligated ions in the +2 and +3 oxidation states. Recent work suggests that Mn(III) may be more toxic than Mn(II), but the reason for this is unclear. Manganese(III) complexes are strong oxidants when compared to Mn(II) complexes, leading to the hypothesis that Mn(III) is more toxic than Mn(II) due to increased oxidative injury. PC-12 rat pheochromocytoma cells were treated with manganese in the +2 [$MnCl_2$] and +3 [$Mn(P_2O_7)_3$] oxidation states. Release of lactate dehydrogenase (LDH) was used to determine cytotoxicity. Measurement of LDH release 24 hours after addition of manganese in the +3 oxidation state revealed an LC_{50} of approximately 400μM. In contrast, addition of the +2 oxidation state produced minimal toxicity at concentrations up to 1600μM. The extent of oxidative injury was determined by HPLC analysis of cellular glutathione and fluorimetric analysis of thiobarbituric acid reactive substances (TBARS). Treatment of cells with 100μM Mn(III) or 800μM Mn(II), approximate LC_{10}'s, significantly decreased cellular glutathione within 1 hour of addition. Four hours after addition of Mn(III), TBARS increased fourfold compared to controls (2.9 *vs.* 0.7 nmol/mg). Cells treated with an equivalent concentration of Mn(II) exhibited control levels of TBARS (0.67 nmol/mg). These data indicate that Mn(III) is more toxic than Mn(II) in PC-12 cells and suggest that Mn(III) toxicity is mediated by the production of oxidative injury.

459 OXIDATIVE STRESS TOXICOLOGY: THE DEVELOPMENT OF A PISCINE MODEL SYSTEM FOR LOOKING AT NUCLEAR FACTOR - KAPPA B RESPONSES.

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NF-κB plays a central role in the regulation of many genes involved in cellular defense mechanisms and immunological responses (e.g. expression of cytokines). Its activation is strongly associated with both the inflammatory response (e.g. macrophage activation) and with cellular oxidative stress. Many of the genes upregulated by NF-κB exert anti-oxidative activity. NF-κB exists in the cytoplasm as a dimer in association with an inhibitory subunit, IκB. Upon activation, IκB is phosphorylated and destroyed by proteolysis, whereupon the transcriptionally active dimer (p50/p65) translocates to the nucleus where it activates transcription of specific genes. Oxidative changes that occur after exposure to chemicals can affect NF-κB. Since it is well understood that exposure to environmental chemicals occurs daily; establishing experimental paradigms for testing environmental chemicals and chemical mixtures for their effects on NF-κB is crucial. To address this issue we are studying the induction and modulation of NF-κB in a piscine cell line and a murine cell line that have been transfected with an NF-κB reporter gene construct. Transfected and control cells are plated in 96 well microtiter plates and allowed to reach 80-90% confluency. Cells are then exposed to 0, 0.001, 0.01, 0.05, 0.1 and 0.5% hydrogen peroxide concentrations alone and in the presence of 10-3-10-6M trolox. The data are consistent for both piscine and murine cell lines in that there is a dose dependent increase in NF-κB reporter gene activity with increasing concentrations of hydrogen peroxide. Moreover, the addition of trolox decreased the induction of NF-κB in a dose-dependent fashion. The findings give further merit to the development of piscine models for exploring effects of environmental chemicals and their mixtures on specific cellular responses associated with oxidative damage.

460 GENERATION OF FREE RADICALS BY INTERACTION OF IRON WITH THIOLS IN HUMAN PLASMA.

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Oxidative stress has been associated with a number of diseases in humans. Among the sources that can generate oxidative stress, it has been reported that iron can generate reactive oxygen species (ROS) with thiol. In iron overload state, increased thiol levels in plasma appeared to be associated with human mortality. In this study we examined whether iron could interact with thiols in plasma, generating ROS. In human plasma, unlike with Fe(¥²), Fe(¥±) increased lucigenin-enhanced chemiluminescence in a concentration-dependent manner, and this was inhibited by superoxide dismutase. Boiling of plasma did not affect chemiluminescence generation induced by Fe(¥±). However, thiol depletion in plasma by pretreatment with N-ethylmaleimide (NEM) decreased Fe(¥±)-induced chemiluminescence significantly, suggesting that Fe(¥±) generated superoxide anion by the nonenzymatic reaction with plasma thiol. Consistent with this findings, albumin, the major thiol contributor in plasma, also generated ROS with Fe(¥±) and this generation was inhibited by pretreatment with NEM. Treatment with Fe(¥±) to plasma resulted in significant reduction of oxygen radical absorbance capacity (ORAC) value, suggesting that total antioxidant capacity could diminished in iron overload state. In conclusion, in iron overload state, plasma may be affected by oxidative stress mediated by nonenzymatic reaction of Fe(¥±) with plasma thiol.

461 ROLE OF OXIDATIVE STRESS IN NICKEL SUBSULFIDE-INDUCED PULMONARY ALVEOLAR MACROPHAGE DYSFUNCTION IN RATS.

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The present study has examined the relationship between nickel subsulfide (Ni3S2)-induced reactive oxygen species, genotoxicity, and cytotoxicity (cell death) using primary cultures of rat pulmonary alveolar macrophages (PAM). Rat PAM in

cultures were exposed to Ni3S2 in suspensions at concentrations 0, 25, 50 and 125 μg/ml for various times up to 48h in the absence, or presence of phorbol myristate acetate (PMA) (1μg/ml). Rat PAM exposed to Ni3S2 demonstrated a dose- and time-dependent increase in PMA-stimulated production of superoxide anion with significant increase occurring only at 125 μg/ml of Ni3S2 at 4h. However, no such significant increase could be seen at lower concentrations of Ni3S2 used, nor at any other later time periods of exposure. Oxidative stress, measured by superoxide anion-dependent reduction of nitroblue tetrazolium, occurred before Ni3S2 at 125 μg/ml produced significant genotoxicity (measured by DNA-protein crosslinks) and cell death (measured by lactate dehydrogenase leakage) in rat PAM. Ni3S2 (125 μg/ml)-induced genotoxicity and cell death were partially prevented by cotreatment with superoxide dismutase (SOD) and somewhat greater protection was shown by concurrent treatment with SOD plus catalase, with concomitant decrease in oxidative stress. Similar partial prevention of Ni3S2-induced genotoxicity and cell death was also noticed due to cotreatments with dimethylthiourea, mannitol and N-acetylcysteine, with concomitant reduction of Ni2+ uptake by rat PAM. The results suggest that reactive oxygen species, such as superoxide anion, hydrogen peroxide and hydroxyl radical may be important mediators of Ni3S2-induced DNA-protein crosslink formation and cell death in isolated rat PAM.

462 S-ADENOSYL METHIONINE (SAM) AND S-ADENOSYL HOMOCYSTEINE (SAH) METABOLISM UNDER VARIOUS STATES OF OXIDATIVE STRESS.

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Oxidative stress in lung cells can alter SAM/SAH ratio. This can profoundly affect cell function and survival. The aim of the study was to determine the effect of various oxidants on SAM/SAH and other sulfur amino acids in A549 cells. Near confluent cells were challenged with sub-lethal oxidants: 1) mM H2O2, 2) enzymatic generation of H2O2 by glucose oxidase (G/GO;5U/ml), 3) 10 mM t-butyl hydroperoxide (t-butylOOH) and 1-5 days exposure to 95% O2 (oxy). Liquid chromatography–mass spectrometry (LC/MS) of SAM and SAH revealed a consistent and marked increase of intracellular SAM under all forms of oxidative stress. SAH was also increased (thus maintaining intracellular SAM/SAH ratio) except after acute treatment with 10 mM t-butylOOH or 5 days oxy, where it was decreased, leading to an increased SAM/SAH ratio (methylation index). Measurement of intracellular cystathionine and cysteine (transsulfuration metabolites) as well as methionine (transmethylation metabolite) by gas chromatography–mass spectrometry (GC/MS) revealed a significant increase under oxidative stress, except with t-butylOOH or 5 days oxy where they remained at control levels. Elevation of SAM was due to an increased activity of methionine adenosyl transferase (MAT) by 2.5- and 3-fold after treatment with t-butylOOH and 5 days oxy, respectively. Under these conditions, DNA methylation status was either unchanged (t-butylOOH) or decreased (5 days oxy). Preservation of SAM/SAH ratio (H2O2, G/GO and 1-4 days oxy) is associated with elevation of metabolites of the transsulfuration pathway which provides cysteine for glutathione synthesis, needed for adaptation to oxidants. By contrast, severe oxidative stress (t-butylOOH and 5 days oxy) caused elevation of SAM/SAH associated with decreased cystathionine and cysteine, indicating decreased utilization of SAM and toxicity.

463 UNCONVENTIONAL FREE RADICAL-DEPENDENT LIGANDS FOR PROSTANOID RECEPTORS.

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Prostanoid receptors are members of the heptahelical G-protein coupled receptor superfamily. The conventional ligands for the known prostanoid receptors have been characterized in detail and are the products of arachidonic acid metabolism. However, experimental evidence suggests that certain oxidized lipids, broadly referred to as the isoprostanes, may also be ligands for the prostanoid receptors. In the present study, we have investigated whether two prototypical isoprostanes (8-iso-$PGF_2\alpha$ and 8-iso-PGE_2) were ligands for the human TP receptor. Two alternatively spliced forms of the human TP receptor, termed the platelet (TP-P) and endothelial (TP-E) type receptors were investigated. Activator protein-1 (AP-1)-luciferase activity (AP-1-Luc) in a transient transfection assay was used as a molecular readout of TP receptor activity. AP-1-Luc activity was increased by a known TP receptor agonist (U46619) in Chinese Hamster Ovary cells (CHO) transfected with the TP-P and TP-E receptors, but not in vector control cells. U46619-mediated AP-1-Luc activity was inhibited by known TP receptor antagonists (ISAP, SQ 29, 548) as expected. Treatment of CHO TP-P and TP-E cells with 1-100 nM 8-iso-$PGF_2\alpha$ was not associated with increased AP-1-Luc activity. In contrast, AP-1-Luc activity was increased in a concentration-dependent fashion by 1-100 nM 8-iso-PGE_2 in TP-P and TP-E cells, and this response was inhibited by cotreatment of cells with a TP receptor antagonist (SQ 29, 548). Cyclooxygenase and thromboxane synthetase inhibitors did not reduce 8-iso-PGE_2-mediated AP-1-Luc activity. Interestingly, AP-1-Luc activity was increased by low (nM) 8-iso-PGE_2 concentration in a TP antagonist independent fashion in naive CHO cells. Collectively, these observations suggest that 8-iso-PGE_2 may be a ligand for multiple receptors and support the notion that oxidized lipids may be unconventional prostanoid receptor ligands (DE-AC06-76RLO to TJW).

464 SPECIES DIFFERENCES IN HEMATOTOXICITY OF N-HYDROXY-4-(DIFLUOROMETHOXY)ANILINE *IN VITRO*.

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Hemolytic anemia and methemoglobinemia are common hematologic findings in animals exposed to arylamines or their metabolic precursors. It is generally accepted that hematologic effects of arylamines are mediated by their *N*-hydroxylated metabolites, which participate in redox cycling in erythrocytes leading to oxidative stress. *N*-Hydroxy-4-(difluoromethoxy)aniline (DFMA-NOH) is a model compound for halomethoxylated *N*-hydroxyarylamine metabolites of agrochemicals. The hematotoxicity of DFMA-NOH was investigated in erythrocytes (RBCs) of rats, dogs, and humans *in vitro*. Methemoglobin (metHb) formation was measured directly, while total oxidized glutathione (GSSX), a biomarker for intracellular oxidative stress was used as an index of hemolytic potential. Concentration-dependent metHb formation was observed in RBCs from all three species over the range 1-150 μM. In the rat, peak metHb levels were observed within 15-30 min, and were maintained for the duration of the experiment (120 min). In contrast, metHb levels increased more gradually in human and dog RBCs, and were still increasing in the dog after 120 min at all DFMA-NOH concentrations tested. The rank order species sensitivity to metHb formation was dog > rat ≅ human, based on area under the response vs time curve (AUC) corrected for vehicle controls. GSSX concentration in RBCs exposed to DFMA-NOH also increased in a concentration-dependent manner in all three species. Peak GSSX concentrations were observed within 15 min after exposure and generally declined throughout the course of the experiment. The decline of GSSX levels was somewhat slower in dog RBCs compared to rat and human RBCs. Based on corrected AUC, the rank order species sensitivity to GSSX formation was dog > rat > human. The results suggest that human RBCs may exhibit equal or lower responsiveness to the hematotoxic effects of halomethoxylated arylamines than common laboratory species used in routine toxicity testing.

465 ALTERED EXPRESSION OF OSTEOPONTIN, ALPHA-1 INTEGRIN SUBUNIT, AND ALPHA-SMOOTH MUSCLE ACTIN IN VASCULAR TISSUE IN RESPONSE TO OXIDATIVE INJURY.

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Allylamine (AAM) has been used experimentally to model human atherosclerosis. Metabolic activation of this aliphatic amine to acrolein and hydrogen peroxide by a vascular-specific amine oxidase is associated with oxidative stress and activation of vascular smooth muscle cells (vSMCs). The adaptive response to repeated cycles of AAM injury involves extensive vascular remodeling and vSMC hyperplasia. vSMCs isolated from AAM-treated rats exhibit proliferative (i.e. atherogenic) phenotypes characterized by increased synthesis and deposition of osteopontin (OPN), altered expression of several integrin subunits, and vSMC dedifferentiation. OPN binds integrin receptors and initiates signaling cascades involved in inflammation, proliferation and migration. Because changes in OPN and surface integrins may represent *in vitro* adaptations of injured vSMCs, studies were conducted to evaluate OPN and integrin expression following repeated cycles of AAM injury *in vivo*. In the present study, six week-old, male Sprague-Dawley rats (175-180g) were treated with AAM (35 mg/kg/day and 70 mg/kg/day) once daily for 20 days to induce atherosclerotic-like lesions in large to medium sized vessels. Immunohistochemical evaluation of frozen sections of rat arteries showed that OPN expression is selectively upregulated at sites of vascular injury/stress. The expression of alpha-1 integrin subunit was also increased in injured vessels. These alterations correlated with marked reduction of alpha-smooth muscle actin staining, consistent with loss of differentiated vSMC phenotypes and extensive cell death. These data are consistent with the hypothesis that OPN and associated adhesion molecules participate in vascular remodeling during episodes of oxidative stress *in vivo*. (Supported by NIH grant HL 22863, HL 62539 and ES 09106).

466 POTENTIAL MECHANISMS OF OXYGEN TOLERANCE IN HUMAN CELLS.

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An oxygen tolerant strain of Hela cells which proliferates even under 80% oxygen, termed 'Hela-80', was derived from wild-type Hela cells ('Hela-20') by selection for resistance to stepwise increases of oxygen partial pressure. We have analyzed these two cell types to determine mechanisms involved in the oxygen tolerance of the Hela-80 cells. Antioxidant defenses such as superoxide dismutases, catalase, reduced glutathione, glutathione reductase and glutathione peroxidase do not differ between the two cell types. We therefore focused on possible differences in intracellular generation of reactive oxygen species (ROS), especially of mitochondrial origin (known to be enhanced by hyperoxia). Intracellular oxidant production, detected *via* oxidation of dichlorofluorescein (DCFH) under normal (20%) and high oxygen (80%) concentrations was significantly (~2-fold) higher in Hela-20 cells compared to Hela-80 cells suggesting that Hela-80 cells produce less ROS. The source of ROS is evidently mitochondrial because in both Hela-20 and Hela-80 cells, the mitochondrial uncoupler carbonyl cyanide m-chloro phenylhydrazone almost totally inhibited ROS production as did the combination of rotenone (complex I) and thenoyltrifluoroacetone (complex I and II inhibitors). These data raise the question of what changes in mitochondrial metabolism might have occurred in the Hela-80 cells to cause this diminished ROS production. We have ruled out major differences in lactic acid production but have preliminary evidence that, paradoxically, Hela-80 cells may have elevated cytochrome C oxidase activity. We are considering two main possibilities: (1)Overall mitochondrial metabolism may be lower in Hela-80 cells. (2)Mitochondrial metabolism may be similar in Hela-20 and Hela-80 cells but the Hela-80 mitochondria may be more tightly "coupled", with a lesser tendency to generate ROS from the electron transport chain. Our preliminary data indicating an elevated cytochrome C oxidase activity in Hela-80 cells may provide some support for tighter coupling of electron transport as a potential mechanism for oxygen tolerance.

467 FREE RADICAL SCAVENGERS PROTECT CULTURED CELLS FROM HYDROGEN SULFIDE EXPOSURE.

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Hydrogen sulfide (H_2S) is well-known as a metabolic toxin that inhibits mitochondrial cytochrome *c* oxidase at micromolar concentrations. However, an accumulating body of evidence suggests that H_2S may exert other toxic effects, and that exposure to H_2S concentrations that are currently considered safe may have long-term deleterious effects on human health. The mechanism for these effects is unknown. Recently, metal-catalyzed H_2S oxidation in seawater has been shown to produce free radicals. If free radicals are also produced by H_2S oxidation in tissues, then free radical damage could be an important factor in H_2S toxicity. To test this, we exposed C6 rat glioma cells in culture to various concentrations and durations of H_2S gas, both in the absence and in the presence of three free radical scavengers: dimethyl thiourea (DMTU; 0.01-1.0 mM), N-mercaptoproprionyl-glycine (NMPG; 0.01-10 mM), and reduced glutathione (GSH; 0.1-50 mM). We found that NMPG and GSH, but not DMTU, protected cells in a dose-dependent manner from a 12-hour exposure to 1 mM H_2S followed by a 12-hour recovery, as indicated both by increased cell survival (quantified using the MTT and neutral red assays) and decreased apoptosis (quantified histologically). Furthermore, H_2S toxicity was enhanced by 10 μM Fe^{2+}, which catalyzes H_2S oxidation, but probably also increases radical production. In the absence of scavengers, the dose-response curve assayed by the MTT assay was left-shifted compared with the neutral red assay, suggesting that mitochondria are specifically affected. GSH in particular may protect cells both by scavenging free radicals and by decreasing ambient H_2S, since glutathione in its oxidized form (GSSG) promotes H_2S oxidation. We propose that reduction of GSSG to GSH can be linked with H_2S oxidation *in vitro*, with GSH scavenging the resulting free radicals, regenerating GSSG.

468 NOVEL MECHANISMS OF SUBLETHAL OXIDANT TOXICITY.

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Aging is the highest risk factor for cancer. Although oxidants are thought to contribute to both aging and cancer, the interplay between oxidative stress, aging and cancer has not been well studied. Human diploid fibroblasts (HDFs) undergo premature senescence in response to mild doses of H_2O_2. Since senescence is relevant to aging *in vivo*, we hypothesize that senescent or senescent-like HDFs function as a tumor promoter. We have employed a classical *in vitro* skin tumor promotion model, which involves measurement of colony formation of 308 initiated mouse keratinocytes. 308 cells form colonies when co-cultured with normal HDFs only in the presence of the tumor promoter TPA. TPA induces an average of 5.75±1.15 colonies formed with an average size of 1.96±1.01 mm^2. When co-cultured with H_2O_2 treated HDFs, 308 cells form 30.3±5.50 colonies with an average size of 8.87±0.73 mm^2. To understand the mechanism behind this phenomenon, we tested whether HDF extracellular matrix (ECM), density of HDFs, the contact between keratinocytes and HDFs, or conditioned medium of HDFs play a role in 308 cell colony formation. The ECM alone was not able to induce 308 cell colony formation. Increasing the density of normal, but not senescent, HDFs was inhibitory to the growth of 308 cells. Contact with normal HDFs, but not senescent HDFs was inhibitory to the growth of 308 colonies. The conditioned medium from prematurely senescent cells results in more 308 cell colony growth compared to that from normal HDFs. We are currently exploring the possibility of gap junctions as an inhibitory factor in the growth of 308 cells by normal HDFs. In conclusion, senescent or senescent-like fibroblasts not only lose contact inhibition on the growth of initiated keratinocytes, but also increase production of secreted factors to enhance the growth of 308 keratinocytes. Proteomics techniques will allow us to determine these factors that may play a role in cancer related to aging.

469 THE DISTINCT ROLES OF MAPKS AND PI 3-KINASE IN OXIDANT INDUCED CARDIOMYOCYTE HYPERTROPY.

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Cardiac hypertrophy develops under many types of pathological conditions. Although it is initially beneficial, sustained hypertrophy is often a transition to heart failure. Oxidants have been shown to play a role in heart disease. However, the molecular events induced by oxidants in cardiomyocytes have not been well defined. Our previous studies have shown that isolated rat cardiomyocytes can survive low or mild doses of oxidants but later increase cell volume and protein content. Oxidants are able to activate phosphatidylinositol 3-kinase (PI3K) and p70 S6 kinase (p70 S6K). Here we tested whether H_2O_2 activated AP-1 transcription factor and the relationship between MAPKs, PI3K and P70 S6K in AP-1 activation and hypertrophy induced by oxidants. Gel shift assays showed that AP-1 binding increased at 30 min and reached a maximum at 4 hrs after treatment. AP-1 luciferase activity increased at two hrs, was sustained for at least 6 hours and returned to basal level 24 hours after a 30 min H_2O_2 exposure. Phosphorylation (activation) of p42/p44ERK, p38 and JNK was induced by H_2O_2 within 10 min. The ERK inhibitor, PD98059 but not the p38 inhibitor SB202190 prevented the AP-1 binding and activation of AP-1. Inactivating PI3K with LY294002 partially inhibited JNK activation but did not affect the activation of ERK, p38 or AP-1. Although PD98059 was able to inhibit H_2O_2 induced phosphorylation of p70 S6K or cell enlargement, SB202190 had a partial inhibitory effect on p70 S6K activity and cell enlargement induced by H_2O_2. Our study indicates while ERKs contribute to AP-1 activation, PI3K and p38 are upstream of p70 S6K and regulate cell enlargement.

470 AMIODARONE AND N-DESETHYLAMIODARONE-INDUCED CYTOTOXICITY IN FRESHLY ISOLATED HUMAN LUNG CELLS.

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Amiodarone (AM), a potent antidysrhythmic agent, can cause serious adverse effects including potentially life-threatening pulmonary toxicity. Vitamin E has been shown to decrease AM-induced pulmonary toxicity in both *in vivo* and *in vitro* animal models. In the present study, we investigated the cytotoxicity of AM and its major metabolite N-desethylamiodarone (DEA), as well as the ability of vitamin E to decrease cytotoxicity of AM and DEA in enriched preparations of alveolar macrophages and alveolar type II epithelial cells freshly isolated from peripheral human lung. Following incubation for 36 h, 100 μM AM and 50 μM DEA significantly decreased ($p<0.05$) mean viability (0.5% trypan blue exclusion) in cell digest as well as in enriched macrophage and type II cell preparations (by 31, 33 and 37 % respectively). Enriched preparations of alveolar type II cells demonstrated AM- and DEA-induced loss of viability earlier (i.e. after 24 h) than macrophages and isolated, unseparated cells (cell digest) (36 h). Also, susceptibility to AM and DEA-induced cytotoxicity differed substantially in cells from different patients. Vitamin E decreased toxicity of AM and DEA by 5 - 10% in cells from two of five different patients. Following incubation for 24 h, intracellular levels of AM and DEA did not differ between preparations enriched in macrophages and type II cells or cell digest, and were not altered by vitamin E. AM-treated alveolar type II cell preparations from two patients produced marginally detectable amounts of an analyte which co-eluted with DEA, suggesting the possibility that human type II cells can biotransform AM to DEA. The results demonstrate that toxicity of AM and

DEA in human lung cells is similar to that observed previously in hamster and rat pneumocytes, and that vitamin E has a slight cytoprotective action in the human cells (Supported by CIHR Grant No. MT-13257).

471 INDUCTION OF FACIT COLLAGENS XII AND XIV DURING BLEOMYCIN-INDUCED PULMONARY FIBROSIS.

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Pulmonary fibrosis is a severely debilitating lung disease that is initiated as a result of many types of severe lung injury. The etiology of the disease includes a thickening of the alveolar wall and a decrease in airspace due to an increase in lung extracellular matrix, particularly type I collagen. Collagens XII and XIV sit on the surface of collagen I fibrils and project outward to interact with other molecules of the extracellular matrix. Recent evidence suggests that these collagens play a role in organizing collagen fibrils and assisting in the maintenance of uniform fibril size. In the present studies we characterized collagen XII and XIV production during bleomycin induced pulmonary fibrosis. We speculate that alterations in the expression of these collagens contributes to the disease. Fibrosis was induced by 4 i.p. injections of bleomycin (1 Unit) every other day into female C57BL mice. Lung samples were collected 2, 4, 8, and 12 weeks after bleomycin or saline (control). The extent of fibrosis was determined microscopically after H & E staining. Tissue expression of collagens I, XII, and XIV was quantified by *in situ* hybridization and immunohistochemistry. A time-dependent increase in collagen I protein was observed in the lungs following bleomycin administration which was first evident after two weeks. Expression of collagens XII and XIV was weak until the onset of fibrosis (4 weeks after treatment). At this time their expression dramatically increased. Whereas collagen XII expression decreased between 8 and 12 weeks after treatment, collagen XIV expression persisted throughout the treatment. Our results are consistent with the idea that collagens XII and XIV play an important role in the organization of the lung extracellular matrix during fibrosis. (Supported by NIH grant HL67708 and NJ Thoracic Society).

472 ANTI-FIBROTIC ACTIVITY OF PIRFENIDONE IN A HAMSTER MODEL OF AMIODARONE-INDUCED PULMONARY TOXICITY.

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Pirfenidone is an agent with demonstrated beneficial effects in some animal models of pulmonary fibrosis and in human fibrotic lung disease. Potentially life-threatening pulmonary fibrosis can be caused by the antidysrhythmic drug amiodarone (AM). We investigated the ability of dietary pirfenidone to ameliorate AM-induced pulmonary damage in the hamster. Intratracheal AM administration (1.83 µmol) increased pulmonary hydroxyproline content (by 22%) and histological disease index values (by 127%), indicative of fibrosis, 21 days post-dosing. Dietary supplementation with pirfenidone (0.5% (w/w) in pulverized rodent chow) for 3 days prior to and continuously after intratracheal AM administration, resulted in pulmonary hydroxyproline content and histological disease index values similar to those for control (water-treated) hamsters. In contrast, starting the pirfenidone supplementation one week after AM administration only partially prevented the pulmonary injury assessed at 21 days post-dosing. The effect of dietary pirfenidone on mitochondrial dysfunction produced *in vitro* by incubation with AM or its primary metabolite, *N*-desethylamiodarone (DEA), was investigated as a possible mechanism of protection. However, the respiratory inhibition and membrane potential disruption of isolated lung mitochondria caused by *in vitro* exposure to AM or DEA were not altered by *in vivo* dietary pirfenidone treatment. These results demonstrate that pirfenidone is beneficial against AM-induced pulmonary fibrosis in the hamster model, but that it does not attenuate the adverse mitochondrial effects of AM or DEA that have been proposed to play an initiating role in AM-induced pulmonary toxicity. (Supported by Canadian Institutes of Health Research (CIHR) grant number MT-13257, and a CIHR Doctoral Research Award to JWC)

473 INHALATION AND INTRAVENOUS PLASMA KINETICS OF VERAPAMIL IN CYNOMOLGUS MONKEYS.

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Administration of drugs by inhalation can result in rapid and efficient absorption, and there is currently great interest in this route as an alternative to parenteral administration. The cynomolgus monkey is generally considered to be a good animal model for man because of its phylogenetic proximity, and because it shows similar metabolism of drugs. In this study we have examined the pharmacokinetics of a model compound after inhalation administration to cynomolgus monkeys. The calcium antagonist verapamil was administered to 2 male and 2 female monkeys by inhalation and by intravenous injection in a cross-over design. For inhalation the drug was administered by mask as a nebulised solution in air at 100 mg/m^3 over a period of 60 minutes, which was estimated to result in a total dose of 1 mg/kg. For intravenous administration, 1 mg/kg was given as a bolus injection. Blood samples were taken before administration and 10, 20, 40, 60, 120, 240 and 480 minutes after the start of inhalation exposure or injection. Plasma levels of verapamil were determined by solid phase extraction and LC-MS-MS as detailed elsewhere (Walles et al., 2001). The pharmacokinetic behaviour of verapamil was modelled on the basis of a two compartment model. The fit to the data revealed the same clearance constants for both application routes: 0.05 [1/min] for compartment 1, and 0.009 [1/min] for compartment 2. These figures are almost identical to those found in a similar study performed with minipigs (Koch et al., 2001). These results support the interest of the inhalation route of administration for pharmaceutical applications and the value of the monkey model.

474 EFFECT OF ACETAMINOPHEN ON NITRIC OXIDE PRODUCTION BY RAT ALVEOLAR MACROPHAGES AND TYPE II PNEUMOCYTES *IN VITRO*.

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Nitric oxide has been implicated in the pharmacological and toxic effects of acetaminophen (APAP). Although recent studies support the hypothesis that APAP may affect the lung, little is known about the mechanism by which this occurs. Because nitric oxide plays a key role in the regulation of pulmonary function and diseases, we have studied the effect of APAP on constitutive and lipopolysaccharide (LPS) - induced nitric oxide production in rat alveolar macrophages (AM) and type II pneumocytes (TII) *in vitro*. Extracellular nitrite levels (a breakdown product of nitric oxide) were measured after 24h culturing in the absence or presence of APAP. Rat alveolar macrophages and type II pneumocytes synthesized significant quantities of nitrite in culture (63 ± 41 and 35 ± 11 nmol/mg/24h, respectively, n=5). A concentration-dependent decrease of this presumably constitutive nitrite production occurred in AM and TII exposed for 24h to APAP (0.1 and 1 mM). Nitrite production decreased by 19 and 25% in AM, and by 20 and 38% in TII, at 0.1 and 1 mM APAP, respectively. LPS (1 µg/ml) stimulated nitrite production in AM (231 ± 31 nmol/mg/24h, $p<0.01$), while TII were less responsive (80± 51 nmol/mg/24h, $p=0.07$). The LPS-stimulated nitrite production in AM and TII was not affected by 0.1 mM APAP, but a significant reduction (by 18%) was observed in AM at 1 mM APAP. Measurements of cell viability (MTT-test) verified that the effects observed were not due to cell toxicity. These observations have demonstrated that APAP decreased the constitutive nitrite production in AM and TII *in vitro*. Because of the important and dual role of nitric oxide in the regulation of pulmonary homeostasis and host defense mechanisms, the relevance of these findings has to be studied in humans. (This work was supported by the European Respiratory Society, FWO-Vlaanderen, and Bulgarian Academy of Sciences.)

475 DETECTION OF m-TOLUALDEHYDE IN TISSUE AND ITS INHIBITION OF PULMONARY CYTOCHROME P450 ISOZYMES.

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The xylenes are amongst the top 50 chemicals involved in industry exposures in the United States. Commercial xylene is a mixture of three isomers, m-, p-, and o-xylene with m-xylene constituting approximately 44% of the total mixture. While the xylenes are not considered particularly toxic, they have been shown to inhibit CYP450 activities in respiratory tissue after i.p. or inhalation exposure. This selective (lung vs.liver) inhibition of CYP450 has been attributed at least partly to m-tolualdehyde (m-ALD), a metabolite of xylene. m-ALD has been detected after intraperitoneal administration of m-xylene or its metabolites concurrent with inhibition of CYP450's 2B1 and 2E1 in lung. The purpose of this study was to determine if m-ALD is detectable in tissues after inhalation exposure to m-xylene or m-ALD. Following exposure, tissues were prepared for extraction using a 2, 4 dinitrophenylhydrazine (DNPH) assay and quantified on reverse phase HPLC using a standard curve of m-ALD. Exposure to 300ppm m-xylene yielded hepatic, pulmonary, blood and nasal mucosa concentrations of 65, 107, 45, and 317-ng/g tissue of m-ALD, as well as CYP450 inhibition. A lower exposure level of m-ALD was used due to the previous observation that this metabolite was more toxic than the parent compound, m-xylene. Following m-ALD exposure at 100ppm, levels of m-ALD in liver, lung, blood, and nasal mucosa were 258, 588, 92, 283-ng/g tissue of m-ALD respectively. Inhalation of m-ALD inhibited pulmonary CYP2B1 and

CYP2E1 as did inhalation of the parent compound m-xylene. The data indicate that the metabolite m-ALD is detectable after inhalation exposure to m-xylene or m-ALD coincident with inhibition of pulmonary CYP450 activity.

476 VANILLOID RECEPTORS MEDIATE CYTOKINE PRODUCTION AND CYTOTOXICITY IN BEAS-2B CELLS.

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The vanilloid receptor type-1 (VR1), or capsaicin receptor, has been implicated as a critical component in the regulation of inflammatory and cytotoxic processes in several different cell types. We have investigated the role of VR1 in mediating the pro-inflammatory and cytotoxic effects of VR1 ligands in BEAS-2B cells. Using RT-PCR we demonstrated the endogenous expression of VR1 in BEAS-2B cells. BEAS-2B cells exposed to capsaicin exhibited dose- and time-dependent increases in their production of IL-6 and IL-8. Resiniferatoxin (RTX) also caused an increase in cytokine production, but anandamide and capsazepine did not. Maximum induction of IL-6 and IL-8 was observed at 24h with 100μM capsaicin. Production of TNFα was not observed. Production of cytokines by BEAS-2B cells was inhibited by modulators of VR1 function; capsazepine inhibited IL-6 production while EGTA, a Ca^{++} chelator, decreased IL-6 production by 43%. Exposure to capsaicin also caused a time- and dose-dependent decrease in cell viability. Similar decreases in cell viability were observed with anandamide and RTX. The LC_{50} for capsaicin, anandamide, and RTX was 100μM, 12μM, and 6μM, respectively. Substance P did not cause cytotoxicity to BEAS-2B cells even though it has been shown to stimulate IL-6 production. Capsazepine (10μM) increased the cytotoxicity of capsaicin by 23%, due presumably to the cytotoxic effect of capsazepine (LC_{50}=25μM). EGTA did not influence the susceptibility of BEAS-2B cells to cytotoxicity. Overexpression of VR1 in BEAS-2B cells also appeared to dramatically increase the susceptibility to cytotoxicity by VR1 ligands. These data suggest that the inflammatory and cytotoxic effects of VR1 ligands in BEAS-2B cells require VR1 binding, but involve separate downstream pathways. This work was supported by the Colgate-Palmolive Post-Doctoral Fellowship in *in vitro* Toxicology and by a grant from the National Institute of Standards and Technology (Contract #6NANBOD0006).

477 BIOCHEMICAL/CYTOLOGICAL CHANGES IN BRONCHOALVEOLAR LAVAGE FLUID IN THE MALE RAT AFTER INTRATRACHEAL DOSING TO 3 DIFFERENT SILICON DIOXIDE POSITIVE CONTROL MATERIALS.

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This study was designed to assess the biochemical/cytological changes in bronchoalveolar lavage fluid (BALF) in the male rat after intratracheal dosing with 3 different silicon dioxide (SiO2) positive control materials (SiO2-Alfa, SiO2-HLS UK and SiO2-US Silica). These evaluations were intended to provide a comparison of the pulmonary toxicologic potential of the positive control materials in order to select the most effective material for future studies and to provide reference data for prior studies. The positive control materials were administered once to Sprague-Dawley rats (10 males/material) at a dose level of 50 mg/kg. In addition, a saline and a Titanium Dioxide (TiO2) negative control material were also administered once to rats (10 males/material) at a dose level of 50 mg/kg. BALF was collected and assayed, for enzyme levels and cytology, on 5 animals/group/interval following a 1-day or 14-day post-dose observation period. At 1 day and 14 days after dosing, changes in BALF results compared to the saline control dosed animals were seen in TiO2 and SiO2 dosed animals. At 1 day after dosing, the responses for the SiO2 dosed animals were greatest for SiO2-US Silica, were intermediate for SiO2-HLS UK and were least for SiO2-Alfa. At 14 days after dosing, the responses for the SiO2 dosed animals were greatest in SiO2-Alfa, were intermediate for SiO2-HLS UK and were least for SiO2-US Silica. The responses at 14 days after dosing were generally less than at 1 day after dosing indicating some recovery from the initial pulmonary effects. The changes in the TiO2 dosed animals were generally the least and were indicative of a nuisance dust response as expected. In conclusion, a comparison of 3 different sources of Silicon Dioxide indicated the US Silica material was most effective for an immediate response, the Alfa material was most effective for a persistent response and the HLS UK material was moderately effective at both time intervals.

478 REPEAT BRONCHOALVEOLAR LAVAGE IN LIVE CYNOMOLGUS MONKEYS.

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Bronchoalveolar Lavage (BAL) is routinely performed at CTBR in rats, guinea pigs, dogs and monkeys at study termination. An investigation was undertaken to assess the feasibility of repeat bronchoalveolar lavage in studies at CTBR, specifically in live cynomolgus monkeys. This procedure could be used in the detection and progression of toxic lung injury, determination of drug concentrations or provide a profile of cell populations. Performing repeat bronchoalveolar lavage on the same day on male and female cynomolgus monkeys required an anesthetic with a short duration of action and no accumulative effect. Following anesthesia, a lavage tube was inserted inside the trachea and directed into a lung until it blocked (secondary bronchia level). The lavage was performed using 0.9 % saline (approximately 5 mL), infused in the lung and then withdrawn. Approximately half of the fluid was recovered. The tube was retrieved slowly and the procedure was repeated in the other lung. Respiration of the animal was monitored and oxygen was available throughout the procedure and recovery period (approximately 2 to 12 minutes) in event of any distress. Repeat bronchoalveolar lavage, in live cynomolgus monkeys did not show significant distress to the animals. Therefore this technique was considered to be a feasible and effective assessment to be included in inhalation toxicity studies.

479 METHACHOLINE AEROSOL-INDUCED NASAL OBSTRUCTION IN THE MOUSE.

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Airway hyperreactivity is often assessed by measurement of the obstructive response to graded concentrations of methacholine aerosol in intact nose-breathing animals. The current study was aimed at determining if increased nasal flow resistance was induced by a typical methacholine exposure protocol. Male C57Bl/6J mice were exposed for consecutive two minute periods to saline, 3, 10, 30, 100 or 300 mg/ml methacholine aerosol, with airway responses being monitored during a four minute period after each aerosol exposure. Intact animal exposures took place in a Buxco double plethysmograph. In intact animals, SRaw was increased by the methacholine protocol to a maximum of roughly 3-fold over the baseline value of ~ 2.4 cm H2O*sec. The response was similar in awake and urethane-anesthetized mice. Nasal responses were assessed by surgically isolating the upper respiratory tract of urethane-anesthetized mice *via* insertion of an endotracheal tube and drawing air through that site at a constant flow rate of 25 ml/min. Using the identical methacholine exposure protocol, nasal flow resistance was increased roughly 3 fold over baseline values of ~ 3 cm H20/ml/sec. The nasal responses mirrored those observed for the intact animal. Moreover, nasal responsiveness appeared to be heightened in mice with ovalbumin-induced allergic airway disease. These results suggest that nasal obstruction may contribute to measurement of airway hyperreactivity by standard protocols in intact mice. This work supported by NIEHS R01-08765 and the University. of Connecticut Pulmonary Research Consortium.

480 IMMEDIATE RESPONSES OF MOUSE NOSE TO INSPIRED IRRITANTS.

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Previous studies have shown rats exhibit an immediate sensory nerve mediated nasal vasodilatory response to several inspired irritants including acrolein and acetic acid. The maximal response to each vapor was observed at exposure concentrations below the RD50. The response occurred in the absence of alterations in nasal flow resistance. The aim of the current study was to assess the immediate nasal responses of the C57Bl/6J mouse to these two irritants for comparison to the rat. The RD50 values for acrolein and acetic acid were first determined in this strain and were 1.85 and 450 ppm, respectively. To allow for continuous monitoring of responses, exposures were performed by drawing irritant laden air (1.1 ppm acrolein or 330 ppm acetic acid) through the surgically isolated upper respiratory tract of urethane-anesthetized mice at a constant flow rate of 25 ml/min with three parameters being monitored during exposure: nasal perfusion rate (as assessed by acetone vapor uptake), nasal flow resistance, and plasma protein extravasation (by Evans blue dye leakage). Acetone uptake rates were significantly increased during exposure to acrolein but not acetic acid suggesting a vasodilatory response to the former but not latter irritant. Both vapors caused an immediate 3-fold increase in nasal flow resistance. Nasal tissue Evans blue dye content was not increased by exposure to either vapor suggesting that tissue edemagensis did not occur. The results indicate that immediate nasal responses occur to inspired irritants in the mouse, but that the pattern of responses appears to differ slightly from those in the rat This work supported by NIEHS R01-08765

481 INTERSTRAIN DIFFERENCES IN AIRWAY RESPONSIVENESS TO METHACHOLINE CHALLENGE OBSERVED IN UNRESTRAINED, BUT NOT IN RESTRAINED, PLETHYSMOGRAPHY OF MICE.

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The measurement of airway responsiveness in laboratory animals is an essential tool to assess alterations in pulmonary physiology. Recently, an unrestrained barometric, whole-body plethysmograph (WBP) that utilizes enhanced pause (Penh) to quan-

tify airway resistance has gained widespread use as a technique to determine airway responses to aerosol challenge with bronchoconstrictive agents. However, results generated with the unrestrained WBP have come under increased scrutiny because airflow in the lung is indirectly measured. To address this problem, mice with known interstrain differences in bronchial reactivity (A/J, BALB/c, CD-1, and B6C3F1) were subject to methacholine challenge while unrestrained in a WBP and while restrained with a rubber neck collar in the widely accepted noninvasive airway mechanics (NAM) plethysmograph for measurement of Penh and specific airway resistance (SRaw), respectively. Animals were placed into the plethysmographs and were then subject to challenge with aerosols generated from isotonic saline (control) and methacholine solutions of increasing concentration (2.5 to 320mg/ml) for 3min. Measurements of airway resistance were recorded and averaged for 3min following each concentration. Methacholine challenge data for the unrestrained WBP confirmed the previously reported observation that A/J mice have enhanced airway responses to cholinergic challenge, demonstrated by a concentration of methacholine required to induce a 200% increase in airway resistance (PC_{200}) that was at least 4-fold lower than that observed in the CD-1, BALB/c, and B6C3F1 strains. In contrast, mice restrained in the NAM did not differentiate this strain difference in airway reactivity, with A/J mice demonstrating PC_{200} values were indistinguishable from those observed in the CD-1 and BALB/c mice. Thus the unrestrained WBP is a more sensitive tool than the restrained NAM in assessing airway resistance and responsiveness to bronchoconstrictive agents.

482 ADAPTATION TO STRESS INDUCED BY RESTRAINING RATS IN NOSE-ONLY INHALATION HOLDERS.

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Nose-only inhalation studies involve restraining rodents in nose-only inhalation holders. Protocols for adapting rodents to restraint are used to minimize the stressful effects but there is limited data on the efficacy of adaptation procedures. We examined 1) whether a gradual increase in the duration of restraint facilitates adaptation, 2) how much time is needed for full adaptation when restraint is repeated daily for 4 hrs/day, and 3) whether adaptation persists when restraint is interrupted for several days. Experiments were done in 3 groups of male Sprague-Dawley rats (n = 5/group) as shown in the Table. Heart rate and body temperature were monitored as measures of stress using ECG transmitters. Restraint of rats in nose only holders caused an increase in heart rate (approx. 40 BPM, $p < 0.05$) with no significant change in temperature compared to cage controls. After the first 4 days, the restraint-induced increase in heart rate was the same in rats that were gradually exposed to restraint compared to rats restrained for 4 hours per day. There was a gradual decrease in the effect of restraint on heart rate. Full adaptation to restraint required 14 days of repeated daily restraint. Adaptation persisted even after an interruption of 4 days. In conclusion, nose only restraint for 4 hours caused a modest increase in heart rate. The rats slowly adapted to the stress of restraint with repeated daily exposure indicating that adaptation to nose-only inhalation holders is not necessary for short term exposures except when heart rate is an important factor. A gradual increase in the duration of restraint does not facilitate the adaptation process. Supported in part by EPA R827351.

Purpose	Day	Group 1	Group 2	Group 3
Gradual vs repeated restraint	1-4	cage only	increase restraint by 1 hr per day	4 hrs restraint/day
Fully adapt groups 2 + 3	5-9	cage only	4 hrs restraint/day	4 hrs restraint/day
Interupt repeated restraint	10-13	cage only	cage only	4 hrs restraint/day
Test effect of interuption	14	cage only	4 hrs restraint	4 hours restraint

483 A METHOD TO CHARACTERIZE AEROSOLS PRODUCED BY VOLUNTARY RESPIRATORY MANEUVERS.

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Many respiratory diseases are associated with changes in the composition and balance of fluids within the airways. The purpose of this study was to develop a system to measure the production of aerosols generated while performing a variety of respiratory maneuvers. Results of these measurements will be used to determine if aerosol generation is associated with airway fluid composition and the development of airway diseases. The system was designed to measure airflow, expired gas volume, and aerosols generated during a variety of ventilatory procedures. It featured a small dead space and was housed in a chamber held at body temperature to reduce the effects of evaporation and condensation. The temperature and humidity of the collected gas was recorded during the course of each experiment. Prior to each measurement, the test subject breathed HEPA filtered air for two minutes to reduce the contribution of inhaled ambient particulates. The subject then voluntarily coughed or performed an exhalation procedure into a mouthpiece attached to a spirometer. The spirometer was used to characterize the airflow pattern while collecting the exhaled air and aerosol. Three types of particle analyzers (APS, GRIMM, SMPS) were used to characterize aerosol size over a range between 10 nm and 10 µm. Results show that aerosol characteristics were consistent for each test subject but varied widely between subjects. This method has potential in evaluating the toxicological effects of substances that alter airway integrity. This project is supported in part by FAA IAG 97-11.

484 CHARACTERIZATION OF RESIDENCE TIMES FOR GASES AND AEROSOLS FLOWING THROUGH CHAMBERS USED TO MEASURE RESPIRATORY PARAMETERS OF RODENTS.

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Inhalation studies commonly use two basic systems to provide respiratory information for mice and rats used in pulmonary toxicity research on airborne agents. Bronchoconstrictive agents can be characterized by measuring the enhanced pause (Penh) in the breathing pattern of an unrestrained rodent in a whole body plethysmograph (WBP). Direct measurements of airflow and volume may be made on a rodent restrained by a rubber neck collar in a noninvasive airway mechanics (NAM) plethysmograph. However, variations in aerosol concentrations in a plethysmograph were observed in a recent study [Bowen, L.E. et al., 2001. Toxicologist, 60]. This variation in aerosol concentration could be due to nonideal air flow patterns in the chamber. The uniformity of flow distribution within a chamber was characterized by the mean residence time for a step change in gas or aerosol concentration. A rat and a mouse WBP, and a rat and a mouse NAM (Buxco Electronics, Inc.,) were evaluated. An atmosphere of the airborne material was introduced into the plethysmograph chamber, allowed to reach equilibrium, and then flushed out with clean air. The mean residence time for mixing and clearance of nitrogen, ultra fine carbon black and latex particles were evaluated for the WBP and NAM chambers and compared with the mean residence times of ideal exposure units of the same volume. Results showed that nitrogen gas diffused in the rat WBP within 17.5 % and the rat NAM within 1 % of the ideal mean residence time. Introduction of ultrafine carbon black or latex particle aerosols resulted in a shorter mean residence time by more than 31 %. These shorter experimental times indicated that the aerosols were, in effect, mixing in a smaller volume of the inhalation chambers, supporting the possibility of non-uniform distribution of particles in the inhalation chambers. Similar results were obtained for the mouse WBP and NAM. Thus, the uniformity of distribution within a plethysmograph should be characterized, especially for short duration inhalation tests.

485 AIR-LIQUID INTERFACE CULTURE FOR EVALUATION OF CELLULAR EFFECTS OF COMBUSTION EMISSIONS.

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Combustion products from mobile sources contribute to a particulate material fraction of air pollution implicated in adverse effects on human health ($PM_{2.5}$). In these studies, we have examined the effects of a major constituent of $PM_{2.5}$, diesel exhaust emissions, on cells in culture under conditions more closely representing the physiology of the intact lung than conventional submersion culture. The Cultex system allows cell exposure to a continuous flow of material at an air-liquid interface in a controlled manner. Prior to this work, no known study of the dose of material deposited from an atmosphere onto the Transwell membrane surface had been conducted. Here we present the deposition dose and deposition homogeneity for a delivered amount of PM and gases to the Cultex system. PM deposition was studied using fluorescent microspheres ranging in size from 50 nm to 5 microns. Gas deposition was measured using NO_2 and the Salzmann method. Both PM (all investigated particle sizes) and gases were homogenously distributed throughout the system. To test the biological effects of diesel exhaust on lung epithelial cells, A549 human type II-like cells were grown to confluence on Transwells and exposed to diesel exhaust at an air-liquid interface using this system. The exhaust was diluted to 1000 mg/m^3 total PM and passed over the cell surface at a flow rate of 8.3 ml/minute per Transwell. Cells were exposed to the exhaust for 1 hr, or to whole exhaust or filtered exhaust for 3 hr. The cells were then returned to conventional submersion culture conditions for 1 or 24 hr, and overt toxicity (LDH release, loss of ATP, or changes in mitochondrial tetrazolium salt cleavage), oxidant stress (glutathione content), epithelial integrity (diffusion of Evans Blue-labeled BSA across

the monolayer), and inflammatory potential (IL-8 secretion, at 24 hr only) were evaluated. These conditions resulted in little overt toxicity, but oxidant stress and increased epithelial permeability were observed. Supported by the Office of Heavy Vehicle Technology, US. Department of Energy

486 EXPRESSION OF 15-LIPOXYGENASE-2 IN THE EPIAIRWAY™ *IN VITRO* HUMAN TRACHEAL/BRONCHIAL EPITHELIAL MODEL: REGULATION BY TNFα AND INF-γ.

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15-Hydroxyeicosatetraenoic acid (15[S]-HETE) is the predominate arachidonic acid (AA) metabolite produced by human tracheal/bronchial tissue. This product of the 15-lipoxygenase (15-LOX) enzyme is believed to play a significant role in human airway inflammation and respiratory diseases including cystic fibrosis and asthma. In the current work, RT-PCR and Western blotting techniques were utilized to investigate the expression of 15-LOX isozymes in EpiAirway™, a commercially available *in vitro* model of human tracheal/bronchial epithelium. The effects of IL-1β, TNFα, the TH2 cytokines IL-4 and IL-13, the TH1 cytokine INFγ and various combinations of these cytokines were examined. The previously reported expression of 15-LOX-1, as well as induction of enhanced 15-LOX-1 expression by IL-4 and IL-13, was confirmed by RT-PCR. Additionally, 15-LOX-2 message and protein were detected in the EpiAirway model. TNFα was found to induce expression of 15-LOX-2 message and protein, while the TH1 cytokine INFγ inhibited the TNFα-induced increase in both message and protein. Identical results were obtained in EpiAirway constructs derived from four separate airway cell donors, including one asthmatic donor. These results demonstrate, for the first time, 15-LOX-2 expression in human tracheal/bronchial epithelial cells. The induction of 15-LOX-2 by TNFα and inhibition by the TH1 cytokine INFγ suggest a possible important role of 15-LOX-2 in mediating human airways diseases such as asthma, which is characterized by increased TNFα and decreased TH1 (INFγ)/TH2 (IL-4, IL-13) ratios.

487 THE INFLUENCE OF RISK COMMUNICATION ON RISK PERCEPTIONS.

C. P. Tabone and R. D. Harbison. *Environmental and Occupational Health, University of South Florida, Tampa, FL.*

This study examined variations in public attitudes and perception bias toward environmental risk. Attitudes and perception are influenced by shared opinions, sometimes based on emotion and rooted beliefs. Information concerning environmental risk is delivered to the public through the media, often diluted or inaccurately portrayed as fact. The mixed messages the public receives through faulty risk communication may be conflicting, creating mistrust and confusion. This may effect the views and attitudes of the public during the process of evaluating risk and creating policies. The purpose of the study was to evaluate the public's perception of environmental risk between two target groups: laypeople and occupationally exposed workers. A survey of adults (N=300) of voting age in the Tampa Bay area was conducted to study the attitudes of the public toward environmental risk. A group of laypeople (N=150) were surveyed to evaluate the significance of fears of environmental exposure and related health risks. A group of people (N=150) who work with occupational risks, such as those employed to manage chemical or physical agents (i.e.: asbestos, radon), were surveyed. Demographic information was collected to determine if public perception of environmental risk might be correlated with specific variables. These variables are: 1) age group (Baby Boomers *versus* non-Baby Boomers), 2) education (college degree *versus* non-degree), 3) parental status, and 4) source of environmental information (news media *versus* technical information). The results of this investigation showed similar overall perceptions of environmental risk between each target group, but with demographic biases within each group. (Supported in Part by ATSDR)

488 DEFINING A METHODOLOGY TO ASSESS THE ENVIRONMENTAL HEALTH STATUS OF A COMMUNITY.

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Evaluating the impact of environmental pollution on the health status of a community has long been a goal of public health practitioners. Recently, several databases that track health-based environmental indicators have become available. The purpose of this study was to develop a health outcome model that would be useful for evaluating the relationship between environmental pollution and disease. Once those indicators were identified, the CATCH (Comprehensive Assessment Tracking Community Health) Methodology was used to assess the environmental health status of Hillsborough County, Florida. The National Emissions Trends database was used to access the 1996 estimates of annual emissions of air pollutants from all sources for Hillsborough County. The environmental indicators selected included carbon monoxide, nitrogen oxides, particulate matter (< 10 microns), particulate matter (<2.5 microns), sulfur dioxide, volatile organic compounds, ammonia, and total industrial releases from the Toxic Release Inventory (TRI). The model compared the above indicators with morbidity data. The CATCH Methodology reveals that the majority of the indicators remain areas of concern for Hillsborough County when their levels are compared to both peer counties as well as the State. This methodology will be useful in helping communities to effectively utilize limited public health resources to formulate efficient interventions and preventive strategies. (Supported in Part by a Grant From ATSDR)

489 EXAMINATION OF HEALTH TOXICITY DATA WITH APPLICATION TO BENEFIT-COST ANALYSIS OF ENVIRONMENTAL HEALTH POLICY.

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Health toxicity data is a key input to analysis of environmental health policies and require careful use and examination of inherent assumptions prior to application in policy analyses. Using a case study of the health benefits derived from Clean Air Act (CAA) regulations, we have focused on two key issues: the lack of transparency of the use of health measures and dose-response in the calculation of health benefits and the clarification of assumptions associated with the use of epidemiological data for benefit-cost analysis. To address the lack of transparency, we developed and applied a computerized template tool, the Fast Environmental Regulatory Evaluation Tool (FERET), to explicitly reference the studies, dose-response parameters, and default assumptions used in the CAA analysis. Calibration to existing analyses showed no difference in the key mortality drivers but differences did exist for morbidity effects. Based on these analyses, key needs in the interface of health research and benefit-cost analysis include explicit definition of the populations at risk, use of study-specific data instead of default assumptions, and increased coordination between health and economic researchers. For example, lack of consideration of multiple simultaneous causes for hospital admission may overestimate total admission estimates by 39-65%. Lack of consideration of hospital readmission may overestimate the affected population by 15-34%. As these impacts are age specific, misestimation of health impacts can be problematic for analyses of sensitive subpopulations. Thus, in this case example, not only can lack of available scientific evidence hinder the economic analysis but inappropriate use of health information can result in misestimation of health benefits associated with environmental improvement. [Funded by the Center for the Study and Improvement of Regulation at Carnegie Mellon University. and the University. of WA.]

490 A RISK/BENEFIT ANALYSIS OF PENTABROMODIPHENYL ETHER.

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Brominated Diphenyl Ethers are a class of Brominated Flame Retardants (BFRs) widely recognized as effectively decreasing the risk of death and injury from fire by reducing the potential for flashover. Statistics indicate that 50–60% of residential fires flashover and spread beyond the room of origin increasing the risk of injury or death. An average of 4, 000 people die each year in residential fires and another 20, 000 suffer serious injuries. This study compares the non-cancer human health risk from environmental pentabromodiphenyl ether (PeBDPE) exposure and the reduction in the risk of injuries and death from residential fires attributed to the use of BFRs. Probabilistic risk estimates of injury and death from residential fires were evaluated using data from the US Census Bureau and the National Fire Protection Association. An estimated 45% reduction in the risk of death and injury due to residential fires can be achieved with the continued and increased usage of BFRs in household materials. An evaluation of the health risks posed by environmental exposure to PeBDPE was conducted by calculating the expected oral and dermal uptake based on concentrations measured from soil, water, sediment, and air, as well as animal tissues and human fluids. Calculated hazard quotients for residential (1.4E-3) and industrial (4.9E-3) scenarios were below 1 indicating no adverse toxic effects are predicted based on EPA recommended toxicity values (RfDs) and the potential worst-case exposure parameters. Thus, environmental exposure to PeBDPE poses no adverse health risk while the benefits of use contribute to a predicted 45% decrease in the risk of death and injury in residential fires in the US

491 DAILY WATER INGESTION IN THE US - UPDATE.

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We have published a report entitled "Estimated Per Capita Water Ingestion in the United States" (EPA-822-R-00-008) which is based on USDA's 1994-1996 data on Continuous Survey of Food Intake by Individuals (CSFII), covering a total of 15, 303 individuals. Water refers to plain drinking water and water added in final preparation of beverages and foods at home, school cafeteria or in a restaurant. The data base on young children was limited. Recently, we obtained the 1998 CSFII data from USDA that focused only on children. These new data are for drinking water ingestion by more than 5, 500 children (from birth through 9 years of age) who were not included in the previous survey. We have revised our previous estimates of drinking water ingestion using the expanded sample for infants younger than 1 year old (from 359 to 1, 486) and for children 1 through 9 year-old (from 3, 986 to 8, 157). The increase in sample size for children enabled us to generate more precise estimates of water ingestion for the children, especially for the new-born to 6-month-old, and the 6-month to 1-year-old groups. We have calculated the per capita estimates of mean and percentile distributions of daily water ingestion for community, bottled, other water and water of all sources by age and gender, based on per person and per kg body weight. The opinions expressed are those of the authors and do not necessarily reflect USEPA policy.

492 HUMAN HEALTH RISK ASSESSMENT FOR n-BUTYL ACETATE.

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A risk assessment to determine acceptable levels of n-butyl acetate in drinking water was conducted according to Annex A of American National Standards Institute/NSF International (ANSI/NSF) Standard 60/61. The scientific literature was reviewed for human and animal data. No oral studies of adequate quality were identified. Thus, the critical study was determined to be a 13-week inhalation study in which rats inhaled n-butyl acetate (0, 460, 1380, or 2760 mg/kg-day in females and 430, 1290, or 2580 mg/kg-day in males) for 6 hours/day and 5 days/week. The dose-related effects in both sexes were sedation and hypoactivity during exposure, and decreased mean absolute spleen weights. At the mid dose, considered the LOAEL in both sexes, significantly decreased mean body and absolute and/or relative organ weight changes were also observed. Thus, the low dose in males, 430 mg/kg-day, was considered the NOAEL. A 14-week neurotoxicity study, employing the same doses and exposure paradigm as the critical study, provided support for the sedation, hypoactivity, and decreased body weight observed in the critical study. Since n-butyl acetate is rapidly metabolized to n-butanol in the blood and brain, the likely mediator of the sedation and hypoactivity caused by n-butyl acetate is n-butanol. Based on a total uncertainty factor of 3, 000x to account for inter- and intraspecies, and subchronic to chronic extrapolation, as well as database deficiencies, the oral RfD was calculated to be 0.1 mg/kg-day. This oral RfD compares favorably to the IRIS oral RfD of 0.1 mg/kg-day for n-butanol. The resulting Short-Term Exposure Level (STEL) is 10 mg/L. Long-term exposure levels derived include a Total Allowable Concentration (TAC) of 1 mg/L, and a Single Product Allowable Concentration (SPAC) of 0.1 mg/L. These drinking water action levels are considered to be adequately protective of human health.

493 HUMAN RISK ASSESSMENT FOR γ-BUTYROLACTONE TO DETERMINE DRINKING WATER ACTION LEVELS.

C. D. Gillilland, G. L. Ball and C. J. McLellan. *Toxicology Services, NSF International, Ann Arbor, MI.* Sponsor: M. Dourson.

A risk assessment to determine acceptable levels of γ-butyrolactone in drinking water was conducted according to Annex A of American National Standards Institute/NSF International (ANSI/NSF) Standard 60/61, since it had been detected as a product extractant. Subchronic and chronic mouse and rat oral studies were evaluated. The key effect observed in these studies was general hypoactivity, with progressively more severe neurotoxic effects observed as the doses increased. The effect was acute and occurred at a lower dose than any chronic effect. Further, the weight of evidence suggested that γ-butyrolactone was not genotoxic. γ-Butyrolactone is rapidly metabolized to γ-hydroxybutyrate. Therefore, neurotoxicity studies on this metabolite were also reviewed. From these, an acute human study in which twenty adult males were administered single oral doses ranging from 35-63 mg/kg γ-hydroxybutyrate was chosen as the critical study. Lethargy was observed at all dose levels, with coma induced in subjects treated with doses of greater than 50 mg/kg γ-hydroxybutyrate. Electroencephalographic patterns similar to slow sleep were observed, in addition to alpha rhythms, in unconscious subjects. Brief delta and theta slowing was observed in subjects who were awake. This study was chosen because it was a human study and because the neurotoxic effects induced by γ-hydroxybutyrate occurred at doses lower than those in the repeated dose animal studies with γ-butyrolactone. This ensures that the resulting action levels would be based on the most sensitive toxicological endpoint. A LOAEL of 35 mg/kg was determined. Uncertainty factors of 10x each for intraspecies variability, database deficiencies, and the LOAEL to NOAEL extrapolation were used to calculate an oral RfD of 0.035 mg/kg-day. The Short-Term Exposure Level (STEL) was calculated to be 1 mg/L, and the chronic exposure levels of the Total Allowable Concentration (TAC) and the Single Product Allowable Concentration (SPAC) were calculated to be 1 mg/L and 0.4 mg/L, respectively.

494 IDENTIFICATION OF COMMON TOXIC EFFECTS WITH COMMON MECHANISMS OF TOXICITY FOR PESTICIDES SELECTED FROM THE DRINKING WATER CONTAMINANT LIST (CCL).

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The Food Quality Protection Act (FQPA) has mandated that the EPA assess the cumulative risks to human health from aggregate dietary and non-occupational exposure to pesticides and other substances with a common mechanism of toxicity. It is necessary to identify chemicals or groups of chemicals that cause a common toxic effect based on a common mechanism of toxicity before assessing the cumulative of exposure to chemical mixtures. In this study, the pesticides identified in the CCL, FR 63(40), 1998, have been evaluated as candidates for cumulative risk assessment. A literature search was performed on MEDLINE, TOXLINE, and other databases using the CAS numbers for 40 candidate pesticides or major degradation products of known toxicological concerns. The extensive volume of retrieved literature, ~ 70, 000 citations with abstracts, was entered on to PNNL's Spatial Paradigm for Information Retrieval and Exploration (SPIRE) software to identify groups of compounds with a commonality of toxic effects and mechanisms of toxicity. These groups of compounds were later sorted based on specific toxic effects, i.e. hepatotoxicity, neurotoxicity, reprotoxicity, etc. The "toxic effects groupings" were viewed with SPIRE's visualization tools to identify pesticides or degradation products likely to possess common mechanisms of toxicity. Once relevant groups were identified, the selected scientific literature associated with each group was retrieved and the information used in the formulation of a table of toxic effects and mechanisms of toxicity common to groups of compounds. This end product may be used as a basis for conducting cumulative risk assessment as needed. DISCLAIMER:The opinions expressed in this study are those of the authors and do not necessarily reflect those of EPA.

495 RISK COMPARISON OF SIX VOLATILE ORGANIC COMPOUNDS (VOCS) DETECTED IN CALIFORNIA DRINKING WATER.

P. R. Williams and P. Sheehan. *Exponent, Menlo Park, CA.* Sponsor: D. Paustenbach.

The California Department of Health Services maintains a drinking water monitoring database that consists of analytical data for more than 450 chemicals. In this paper, we evaluate the detection frequencies, average concentrations, and relative health risks of six VOCs in California drinking water that are classified as "known" or "potential" human carcinogens. Although not formally classified as a human carcinogen by the USEPA, methyl tertiary butyl ether (MTBE) is included in the analysis, given its recent controversy in California. We find that from 1995 to 2000, the percentage of drinking water sources containing at least one chemical detection is about 17% for chloroform, 9% for tetrachloroethylene (PCE), 8% for trichloroethylene (TCE), 2% for 1, 1-dichloroethylene (1, 1-DCE), 1.7% for MTBE, and <1% for benzene. Interestingly, the detection frequency for each VOC has not changed much over the last six years. Average detected concentrations from 1995–2000 are about 15 parts per billion (ppb) for PCE and TCE, 10 ppb for chloroform and MTBE, and 4 ppb for 1, 1-DCE and benzene. Average detected concentrations have also remained relatively stable over the last few years, but exceed California's Maximum Contaminant Levels (MCLs) for benzene, PCE, and TCE. To compare the relative risks posed by these VOCs in drinking water, estimates of lifetime cancer risk were also calculated for each chemical based on drinking water exposures *via* ingestion, inhalation, and dermal routes, and using average exposure parameter values and California-derived cancer slope factors. Estimated cancer risks are the greatest for 1, 1-DCE, followed by PCE, benzene, chloroform, TCE, and MTBE. When adjusted for frequency of detections, PCE accounts for the greatest risk, followed by chloroform, 1, 1-DCE, TCE, benzene, and MTBE. The results of this analysis provide useful information on the incidence and relative risks of different drinking water contaminants in California, which can be used to prioritize public health hazards and aid in the development of appropriate risk mitigation efforts.

496 DERMAL EXPOSURE TO CONTAMINANTS WHILE SWIMMING: AN ASSESSMENT OF THE RISKS AND HAZARDS ASSOCIATED WITH USEPA AMBIENT WATER QUALITY CRITERIA.

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Currently, Ambient Water Quality Criteria (AWQC) based on protection of human health consider intake from fish ingestion and, if the water is designated as a potable water source, from direct ingestion. Intake from dermal contact while swimming is not included in the development of these risk-based criteria. In order to evaluate the impact of this omission on the protectiveness of the AWQC, risks and hazards from dermal contact while swimming in waters with chemicals at AWQC concentrations were calculated. The assessment was focused on 42 carcinogens and 27 non-carcinogens for which AWQC are calculated based only on human health risks. For each chemical, Monte Carlo simulation was used to assess the variability of intake and risks among swimmers. Body weight data from NHANES 3 were entered as a custom distribution, and surface areas were calculated from allometric scaling based on body weight. A custom distribution for swimming frequency was taken from the 1994-95 National Survey on Recreation and the Environment. Swimming duration was entered as a triangular distribution with a minimum of 10 min, a maximum of 5 h, and a most likely value of 1 h. Dermal absorption was assessed using a non-steady state approach, given the limited duration of contact per each swimming event. For carcinogens, an exposure duration of 70 yr was assumed, and cancer slope factors were corrected for body weight. Among carcinogens, risks from dermal exposure for individuals at the 95th percentile ranged up to about 10^{-3}, depending upon the chemical. For non-carcinogens, 95th percentile hazard quotients for dermal exposure ranged up to values well over 100. For some chemicals, virtually all swimmers in waters at the current AWQC would have an excess cancer risk $>10^{-6}$ or a HQ >1 from dermal exposure only. The results of this analysis suggest that dermal intake should be considered explicitly when developing surface water criteria for waters where recreational swimming occurs.

497 DETERMINATION OF A HUMAN ACUTE NO-OBSERVED-ADVERSE-EFFECT LEVEL (NOAEL) FOR COPPER IN BOTTLED DRINKING WATER: VOLUME, DOSE AND CONCENTRATION CONSIDERATIONS.

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A human, acute NOAEL for nausea was determined in a prospective, double blind controlled study. As part of an ongoing protocol, Phase II of this study recruited 70 adult females (18-60 years of age) at each of four different international sites (final pooled n = 269). The study design was a 3 x 3 factorial (volume x dose) for 100, 150, or 200 ml of bottled drinking water for 0.4, 0.8, 1.2 mg of Copper (Cu) as sulfate. Two additional doses (0 and 1.6 mg Cu) were added at the 200 ml volume to determine a Dose-Response and corroborate previously reported results. Each subject was given a blind, randomly selected concentration of Cu once a week. Following dosing, all subjects were monitored by health care personnel for 1 hour. All subjects completed a questionnaire at 0, 0.25, 1, and 24 hours post-dosing that screened for positive GI effects (nausea, vomiting, abdominal pain, and diarrhea). Nausea was the most prevalent symptom reported and was generally reported within the first 15 minutes (water volume $p < 0.032$, copper dose $p< 0.0001$ and water x copper interaction is $p<0.97$). As volume increased, the effect of Cu-induced nausea decreased; as Cu dose increased; the incidence of nausea increased. At 200 ml, a significant increase in reported incidence of nausea at 0.25 hr occurred at 0.4 (6 mg Cu/L), indicating a NOAEL of 0.8 mg Cu (4 mg Cu/L) for adult females. An acute nausea threshold of 5.3 mg Cu/L can be determined from the factorial analysis (0.8 mg Cu in 150 ml). These data confirm a previously determined human acute NOAEL for Cu and provide additional, controlled human data for determining regulatory contaminant levels of Cu for drinking water.

498 SCIENTIFIC CRITERIA FOR DERIVING OCCUPATIONAL EXPOSURE LIMITS FOR METALS AND OTHER COMPOUNDS RELATED TO MINING OPERATIONS.

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Despite general agreement that occupational exposure limits (OEL) play an important role in protection of worker health, significant disparities often exist in specific OEL values for a particular substance. These differences often reflect diversity in scientific criteria used among OEL-setting organizations. We investigated areas of scientific consensus and disagreement by conducting a critical evaluation of OEL documentation from various international organizations for a series of five case-study compounds: chromium, copper, lead, manganese, and silica. These chemicals served as a backdrop for evaluating key scientific criteria for setting OELs. As just one example, in the context of OELs for chromium compounds we present a decision logic for evaluating chemical speciation, demonstrate the impact of using particle dosimetry to calculate human equivalent concentrations from animal studies, and recommend approaches for considering nutritional essentially. Some issues became apparent only by comparing all of the case-study substances. For example, we found large differences in the extent of the supporting databases, but no explicit approach for documenting confidence in the resulting OELs. We also evaluated the application of uncertainty factors among various organizations and found that the rationale for their use, areas of uncertainty considered, and recommended defaults differed widely among organizations. Although in many cases we found that equally valid alternative approaches were used to address the scientific issues we explored, the one over-riding theme in our analysis was the need to increase the degree to which the OEL documentation described the selected approach. The results suggest the opportunity for greater scientific harmonization in setting OELs, with the goal of increasing transparency in OEL derivation, and enhanced sharing of resources among organizations that set OELs. Sponsored by the International Council on Metals and the Environment.

499 DEVELOPING ENVIRONMENTAL CRITERIA BASED ON THE ACUTE TOXICITY OF LEAD.

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Effects from chronic lead exposure are a significant public health problem, and the development of environmental criteria for lead have understandably focused on circumstances of prolonged or repeated lead exposures. Occasionally, however, environmental problems arise where the potential for effects from acute lead exposure must be considered. Currently, no criteria are available for this type of exposure in environmental settings. In an effort to explore development of such criteria, the toxicological literature was evaluated with the objective of identifying blood lead concentrations that might serve as an upper limit to prevent acute lead poisoning. One approach to setting this limit would be to use the lower end of the range of blood lead concentrations observed in child and adult patients with symptomatic acute lead toxicity. A strength of this approach is that it deals with endpoints of obvious clinical relevance. An alternative approach would be to use the lower end of the range of lead concentrations associated with changes in heme metabolism (e.g., δ-aminolevulinic acid dehydratase, ALAD) as a sensitive endpoint. In basing the blood concentration limit on a sensitive biochemical effect of lead, this approach is likely to be protective for subtle acute effects of lead that might be missed in acutely poisoned patients. Blood concentration limits based on both approaches were compared to predictions of blood concentrations after various acute lead doses using the Leggett model. The Leggett model was used because the IEUBK model is a poor predictor of blood lead concentrations resulting from acute exposure. With the Leggett model, upper limits on lead concentrations in environmental media could be calculated such that acute-toxicity-based blood lead concentration limits are not exceeded. This technique appears to hold promise as a method for developing environmental criteria based on acute lead toxicity.

500 EVALUATING LEAD EXPOSURE AT MINING SITES WITH HETEROGENEOUS SOIL TYPES AND LEAD BIOAVAILABILITY.

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Risk assessments conducted on residential areas near mining and smelting sites typically apply USEPA's Integrated Exposure Uptake Biokinetic (IEUBK) model for children to evaluate lead exposure. The model is designed to estimate blood lead levels, using input assumptions about exposure to lead from various environmental media. Soil lead concentration and bioavailability are among the parameters with the most influence on blood lead estimates. At many mining sites soils are highly heterogeneous with regard to lead concentration, soil chemistry, and the associated soil lead bioavailability. This is due to both the natural mineralization of the area and the variable enrichment from mining activities. The IEUBK model is designed to accept only single input values, usually averages, for each parameter. In heterogeneous settings, use of independent averages for lead concentrations and bioavailability in soil inaccurately reflects lead exposure from either individual samples or from the site as a whole. This study describes application of a soil lead concentration-bioavailability weighted averaging method for deriving input parameters to the IEUBK model at a mining site in comparison with results derived using independ-

ent averages or model defaults. Lead bioaccessibility testing of site soils indicated lead bioavailability ranging from less than 2.5 percent to 22.5 percent. Relative to the independent averaging method, use of the weighted averaging method resulted in a 24 percent lower estimate of geometric mean blood lead and a 70 percent lower estimate of the percent of individuals exceeding the regulatory blood lead limit of 10 micrograms per deciliter. Differences compared to the model default bioavailability factor of 30 percent were greater. Use of the soil lead bioavailability weighted averaging method provides a site-specific refinement to IEUBK model input parameters that improves the model's ability to accurately estimate lead exposure.

501 ASSESSING BIOAVAILABILITY AND OTHER PARAMETERS INFLUENCING EXPOSURES TO ARSENIC ASSOCIATED WITH CCA-TREATED WOOD.

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Potential health risks associated with arsenic originating in wood treated with chromated copper arsenate (CCA) are receiving increasing regulatory, scientific, and public attention. A recent risk assessment found that incidental ingestion of arsenic that may be present on the surface of treated wood structures (termed dislodgeable arsenic) was the primary contributor to exposure associated with arsenic from CCA-treated wood. To evaluate this exposure pathway, several exposure parameters were derived that have not been frequently used in other risk assessment contexts, i.e., the concentration of dislodgeable arsenic on the wood surface (C_{da}), the relative bioavailability adjustment (RBA) factor for ingested dislodgeable arsenic, and the hand transfer efficiency (HTE) factor describing the amount of arsenic that would be removed from the wood surface and subsequently ingested. Parameter estimates were derived based on data from the published scientific literature and other relevant studies. In addition, a sensitivity analysis was conducted to examine the variability and uncertainty of each parameter and the influence of parameter choices on risk estimates. Data sources reviewed in this work included studies of arsenic loadings on hands or wipe materials, studies of arsenic bioavailability from CCA-treated wood and other solid media, information regarding the CCA wood treatment process, studies of soil adherence to hands, skin surface area data, and soil ingestion rate data. The results of the sensitivity analyses indicate that potential values for C_{da} could span the widest range (with the 5th and 95th percentile values for a wood sample differing by as much as a factor of 25). By contrast, the high- and low- end estimates of HTE values differed by a factor of 14, while those for the RBA factor differed by only a factor of 2.3. These results suggest that actual arsenic exposures and risks are more likely to be less than the best estimates calculated in the risk assessment than to exceed them.

502 EVALUATION OF HUMAN HEALTH RISKS FROM EXPOSURES TO ARSENIC ASSOCIATED WITH CCA-TREATED WOOD.

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We performed a human health risk assessment (HHRA) to evaluate exposures to arsenic associated with chromated copper arsenate (CCA) treated wood. Central tendency exposure (CTE) and reasonable maximum exposure (RME) parameters were used to quantify exposures for a residential and a playground exposure scenario. The residential scenario included a male/female child ages 2-6 years, and a male/female child and adult ages 2-31 years. The playground scenario included a male/female child ages 2-6 and 7-12 years. Both exposure scenarios evaluated incidental ingestion and dermal contact with dislodgeable arsenic, which is arsenic on the surface of CCA-treated wood that can be removed by dermal contact with the hands. Eight different samples of treated wood were included in the evaluation. Exposure to arsenic in soil *via* incidental ingestion, dermal contact, and inhalation was also evaluated. The primary source of arsenic in soil was assumed to be a CCA-treated structure where CCA migrated *via* rainwater run-off from the treated structure to the soil below. Estimated cancer and non-cancer health risks ranged from 4.9E-07 to 5.4E-06 and 2.6E-04 to 2.8E-02, respectively using CTE parameters; and 1.4E-06 to 1.2E-05 and 6.9E-04 to 6.4E-02, respectively using RME parameters, depending on wood sample and receptor. For all of the scenarios evaluated, 80 to 90% of the risks were from ingestion and dermal exposures to dislodgeable arsenic. The highest estimated risks were for a resident exposed to a wooden structure made of CCA-treated Southern Pine with pressure-applied water repellent. Estimated cancer and non-cancer health risks from the most commonly used type of treated wood in the US (i.e., CCA-treated Southern Pine), ranged from 1.1E-06 to 6.6E-06 and 6.9E-04 to 3.3E-02, respectively, for the resident. Based on the results of the HHRA, exposures to arsenic from CCA-treated wood in both a residential and playground setting does not pose a significant health risk to children or adults.

503 THE USE OF A WEIGHT OF EVIDENCE APPROACH TO ASSESS HEALTH RISKS FROM ARSENIC EXPOSURE.

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The importance of considering a weight of evidence approach when evaluating arsenic at contaminated sites became clear during two recent projects. Elevated levels of arsenic were encountered in two communities in Ontario, one near a former gold mine and the other near a sintering plant. Risk assessments, involving multi-pathway exposure assessment (air, water, soil, backyard produce, fish and market basket foods) and use of the USEPA slope factors revealed risk levels in the 1 in 1000 range. Ontario considers 1 in 1, 000, 000 to be the acceptable risk level. These elevated risk estimates raised community concerns. Answers were needed to ensure public safety and satisfy community and regulator concerns. Further investigation into the risk assessment revealed the following: (i) market basket foods were the main contributor to arsenic related risks; (ii) generic criteria in Ontario (25 ppm) result in elevated risk levels (greater than 1 in 100, 000); (iii) the contribution of soil to overall arsenic related risks was small; all other pathways were less significant; (iv) health based intervention levels, as determined by the risk assessment, proved to be economically and technologically impossible; and, (v) removal of all soil above the generic criteria would result in a 2 to 4% overall risk reduction. It became clear that information beyond that typically contained within a risk assessment was needed to complete the decision making process. Therefore, limited health status studies were conducted which considered the incidences of cancer in the communities in question. In addition, urinary arsenic studies compared the 'impacted' communities with 'control' communities. In all cases, these studies indicated that the 'impacted' communities were similar to the control communities. Only after consideration of all pieces of evidence (risk assessment, community health status, urinary arsenic study) was it possible to conclude that there were no unsafe exposures or adverse health effects associated with elevated arsenic levels in both communities.

504 COMPARISON OF THE TOXICOLOGICAL PROFILE DETERMINED IN THE SPRAGUE-DAWLEY RAT FOLLOWING DERMAL OR DIETARY EXPOSURE TO A PROSPECTIVE INSECT REPELLANT ACTIVE INGREDIENT(KBR 3023).

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The general toxicological profile of -(1-methyl-propoxycarbonyl)-2-(2-hydroxyethyl)-piperidine (KBR 3023), a prospective new insect repellant active ingredient intended for human use, was studied in rats using the dermal and dietary route of exposure. Dermally applied dosages of 0, 80, 200, 500, or 1000 mg/kg/day were evaluated for 13 weeks (followed by a recovery period); dermal dosages of 0, 50, 100, or 200 mg/kg/day were evaluated for 1 and 2 years. Dietary dosages of 0, 100, 150, 300, or 1, 000 mg/kg/day were evaluated for 2, 5, and 14 weeks. With the exception of lesions occurring at the dermal dose site, the toxicological response of the rat following continuous and repeated dermal or dietary exposure to the test substance for durations of up to 14 weeks, was principally characterized by structural and/or functional alterations in liver- and kidney-related endpoints. Lesions noted in the liver were indicative of an adaptive response to an increased need to metabolize and excrete an exogenous chemical. Lesions observed in the kidney were consistent with an α_{2u}-globulin-induced nephropathy, a common lesion in the male rat. By the end of the four-week recovery period that followed the 13-week dermal exposure, all changes attributable to exposure to the test substance had returned to within normal limits. Results following 1 and 2 years of continuous dermal exposure (excluding dose site lesions) were limited to an increased incidence of areas of cystic degeneration of the liver noted in males sacrificed at 2 years; indications of altered liver function were not observed. The same lesion was not identified histopathologically at 1 year. No evidence of a compound-induced neoplasia was suggested. A comparison of the results of these investigations indicate a very similar systemic toxicological response in the rat following exposure to KBR 3023, regardless of the route of exposure.

505 DIFFERENTIAL SPECIES SENSITIVITIES AND DURATION-SPECIFIC REFERENCE CONCENTRATIONS (RfCs) FOR HYDROGEN SULFIDE (H_2S) EXPOSURES.

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H_2S is an extremely hazardous gas and is the most common cause of death in the workplace. Although information on the human toxicity of H_2S is abundant, exposure concentrations and durations are not well defined. Consequently, regulatory

agencies have had to use available animal data to derive RfCs. A subchronic mouse inhalation study resulted in a LOAEL of 111 mg/m^3 and a NOAEL of 42 mg/m^3 for respiratory effects. Both MADEP and the USEPA derived a chronic RfC of 1 µg/m^3 from the above mouse study using the NOAEL. A subchronic RfC of 10 µg/m^3 can be developed using the same data. Because of the emergence of short-term human exposure data acute RfCs were developed and compared with the RfCs based on mouse data. Mild asthmatics exposed to 2.28 mg/m^3 H_2S for 30 minutes sensed odor, and experienced nasal and pharyngeal dryness, bronchial obstruction and headache. This acute human LOAEL is 40 times lower than the mouse subchronic LOAEL (111 mg/m^3) identified in the study that was used to derive the chronic RfC. A 30-minute RfC of 93 µg/m^3 was developed by applying a safety factor of 30 (10 for the use of a LOAEL and 3 to protect severe asthmatics) to the LOAEL (2.78 mg/m^3). In a separate study, field workers exposed to 125 µg/m^3 H_2S for 5 hours sensed persistent odor, and experienced eye and throat irritation, headache and nausea. If this LOAEL (125 µg/m^3) is adjusted by a safety factor of 100 (10 to account for sensitive individuals, and 10 for LOAEL to NOAEL extrapolation), the 5-hour RfC will be 1.25 µg/m^3: similar to the chronic RfC and lower than the subchronic RfC. In conclusion, the emerging data suggest that: (1) humans may be more sensitive than mice to H_2S toxicity; (2) the current chronic RfC may not be health protective and the interspecies extrapolation factor used to derive the chronic RfC should be reassessed to reflect current toxicity data; and (3) future H_2S toxicity studies should be conducted in H_2S-sensitive species like rabbits and guinea pigs that are identified in the literature. (Abstract may not reflect MADEP policy).

506 UTILIZING DATA FROM MULTIPLE STUDIES (META-ANALYSIS) TO DETERMINE EFFECTIVE DOSE LEVELS: EXAMPLE OF MORTALITY IN RATS AND MICE EXPOSED TO HYDROGEN SULFIDE.

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Most chemical-specific risk assessments use a single best study to identify a toxicity marker, such as a NOAEL, benchmark dose, or LD50. The objective of this exercise is to incorporate as much data as possible from multiple studies to derive a chemical-specific dose-duration response curve from which to identify toxicity markers. This has the advantage of incorporating more information than single-study assessments. The current example uses mortality for rats and mice, analyzed separately, from acute exposure to hydrogen sulfide. Methods of statistical analysis are used to determine when data from different studies can be pooled. EC01, EC10, EC50 (concentrations at which there are response rates of 1%, 10%, and 50%) are estimated with 95% confidence intervals, at durations of 5, 10, and 30 minutes, and 1, 2 4, and 6 hours. A concentration-response curve for mortality is fit to the rat data simultaneously for exposures of 5 min., 10 min., 30 min., and 1 hour, using a logistic curve additive in log(concentration) and log(duration). Separate curves are fit at 2, 4, and 6 hours. Fewer data are available for mice but fits are obtained for 30 minutes and 1 hour. The curves for rats fit the data exceedingly well and exhibit a threshold-like response followed by a steep incline as concentration increases. The response curves for mice start upward at a much lower concentration than for rats and then incline more gradually. Response is clearly more variable in mice than rats. The data on a mix of nonlethal adverse effects at the durations described above, and the observed NOAELs for mortality from individual studies, are plotted by concentration and described in the text. The NOAELs for mortality from individual studies fall within 95% confidence bounds for the EC01 (except lower at 5 minutes for rats and higher at 30 minutes for mice). Nonlethal adverse effects occur at concentrations below the lower bound for EC01 mortality at most durations.

507 THE ORAL REFERENCE DOSE FOR ETHYLENE DIBROMIDE: A NEW LOOK AT AN OLD PROBLEM.

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Previous evaluations of the experimental toxicity data for ethylene dibromide (EDBr) have concluded that limitations of the available studies precluded the derivation of oral toxicity values. Therefore, the USEPA has no oral RfD on IRIS and ATSDR has not developed an MRL for EDBr. Upon critical re-examination of the data, however, with consideration of the strengths and limitations of the existing studies, as well as a proposed mechanism of action for the testicular effects of EDBr, a provisional oral RfD for EDBr of 9x10^{-3} mg/kg-day is presented here. A lifetime carcinogenicity study in rats and mice was performed by the NCI, but was terminated prematurely because the MTD was apparently being exceeded. Even with early termination, dose-related increases in testicular atrophy were noted histologically, as well as increased incidence of tumors of the tunica vaginalis (the serous covering of the testes). Several studies in bulls exposed orally to low doses of EDBr also showed testicular atrophy, which was reversible upon cessation of exposure. Rodent studies of 90 days and 17 months have also examined the oral toxicity of EDBr. The provisional RfD is based on testicular atrophy in rats observed in the NCI study rather than on the effects observed in bulls at lower doses. The rationale for the choice of critical study includes an allometric argument as well as a mechanistic one.

508 NOAEL/LOAEL AND BENCHMARK DOSE APPROACHES TO SETTING ACCEPTABLE HUMAN EXPOSURE LEVELS TO 1, 2-DICHLOROETHANE (ETHYLENE DICHLORIDE).

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Chemical risk assessment approaches other than the most common NOAEL/LOAEL approach are being considered by the EPA. The benchmark dose (BMD) approach has a number of advantages to the traditional NOAEL/LOAEL approach, including not being limited to the experimental doses, and responding to sample size and the associated uncertainty. Because EPA's IRIS database contains no reference dose for chronic oral exposure (RfD) for 1, 2-dichloroethane, multiple approaches were compared for derivation of a provisional toxicity value. The majority of 1, 2-dichloroethane released into the environment enters the atmosphere from its production and use as a chemical intermediate, solvent, and use as a lead scavenger in gasoline. Major human exposure is from urban air, drinking water from contaminated aquifers and occupational atmospheres. Animal studies point to the kidney and liver as target organs for 1, 2-dichloroethane toxicity, consistent with reported injury to these organs in humans following accidental acute ingestion. The principal study selected as the basis for RfD derivation was conducted in rats and used five concentrations in drinking water, ranging from 500-8000 ppm, and a vehicle control. The lowest exposure level was considered a minimal LOAEL for increased kidney weight. Because the principal study examined a sufficient number of exposure levels, an RfD was also calculated using a BMD approach. RfD comparisons were based on several endpoints including increased kidney and liver weights, renal and liver histopathology, and altered enzyme activities.

509 HEALTH RISK ASSESSMENT FOR TRICHLOROACETIC ACID.

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TCA is one of the five haloacetic acids considered for regulation under EPA's proposed Stage 2 Disinfectants and Disinfection Byproducts Rule. A health risk assessment was conducted based on available studies. TCA induces systemic, noncancer effects in animals that can be grouped into three categories: alterations in intermediary carbohydrate metabolism, liver toxicity and developmental toxicity. The liver is the primary target organ for TCA toxicity. In a 104 week drinking water study in male F344 rats (DeAngelo et al., 1997), there was a significant decrease in body weight(10%), minimal histopathological changes in the liver, increased serum alanine aminotransferase level, and increased peroxisome proliferation at 364 mg/kg-day. The No-Observed-Adverse Effects-Leved (NOAEL) is 32.5 mg/kg-day. TCA increased resorptions, decreased implantations, and increased fetal cardiac malformations when administered to pregnant rats at 291 mg/kg-day on gestation days 1-22 (Johnson et al., 1998). In another study (Smith et al., 1989), decreased fetal weight and length, and increased cardiovascular malformations were observed when pregnant rats were administered 330 mg/kg-day TCA by gavage during gestation days 6 to 15. A RfD for TCA of 0.03 mg/kg-day is derived from the NOAEL of 32.5 mg/kg-day for liver histopathological changes identified by DeAngelo et al. (1997). An overall uncertainty factor of 1000 is applied to the NOAEL to calculate the RfD. Mutagenicity data have provided mixed results. TCA was reported to induce liver tumors in mice but not in rats. EPA has classified TCA as Group C: possible human carcinogen. Under EPA's Draft Guidelines for Carcinogen Risk Assessment, TCA can be described as having "Suggestive evidence of Carcinogenicity, but not Sufficient to Assess Human Carcinogenic Potential". (The opinions expressed in this abstract are those of the author and not necessarily those of EPA.)

510 RE-EVALUATION OF THE ORAL REFERENCE DOSES FOR THE BROMINATED TRIHALOMETHANES BROMODICHLOROMETHANE, DIBROMOCHLOROMETHANE, AND BROMOFORM.

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The brominated trihalomethanes (BTHMs) bromodichloromethane (BDCM), dibromochloromethane (DBCM), and bromoform are by-products of drinking water chlorination. Information on the hazard, dose-response, and risk characterization

of these chemicals is being updated. Oral Reference Doses (RfDs) for BDCM, DBCM, and bromoform have previously been derived using the conventional NOAEL/LOAEL approach and are reported in the Integrated Risk Information System (IRIS). The RfDs obtained were 0.02 mg/kg-day for BDCM, based on lesions in the liver, kidney, and thyroid of exposed mice; 0.02 mg/kg-day for DBCM, based on absence of lesions in exposed rats; and 0.02 mg/kg-day for bromoform, based on absence of effects in exposed rats. For the updating process, new health effects data published between 1993 and July, 2001 were identified and evaluated. Toxicological endpoints from new and existing studies were reviewed for suitability for benchmark dose modeling. Benchmark doses (BMDs) for selected endpoints were obtained using the Benchmark Dose Modeling Software (Version 1.2) developed by the USEPA National Center for Environmental Assessment. $BMDL_{10}$ values (the 95% lower confidence limit on the BMD) were used to calculate a new RfD for each BTHM. The uncertainty factors used in derivation of the RfD values were also re-evaluated in consideration of new studies added to the databases for individual BTHMs. Using this approach, the resulting RfD values are 0.003 mg/kg-day for BDCM, based on fatty degeneration in the liver of rats; 0.02 mg/kg-day for DBCM, based on fatty changes in the liver of rats; and 0.009 for bromoform, based on hepatocellular vacuolization in the liver of rats. (This work was conducted under contract to EPA, but the opinions expressed in this abstract are those of the authors and do not necessarily represent EPA policy.)

511 UPDATED ASSESSMENT OF HEALTH EFFECTS FROM TOLUENE EXPOSURE.

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Toluene is widely used in industry as a solvent, gasoline additive, and in polymer production. The present EPA assessments for toluene on IRIS were verified between 1987 and 1990. Since the verification dates, a number of new studies have been published, warranting a reconsideration of the data for toluene. The existing IRIS RfD is based on hepatic effects in rats exposed by gavage. New oral studies not described on IRIS examined hepatic, immunological, and neurological endpoints, and raise the issue of whether or not to change the critical effect for RfD derivation from hepatic effects to one based on immunological or neurological endpoints. The existing RfC is based on a LOAEL for neurobehavioral effects in a study of toluene-exposed electronics workers, but a number of new studies examining neurologic effects in occupationally-exposed humans have been published, including two identifying NOAELs. The new studies support neurological effects as the critical effect for RfC derivation and add more information about exposure-response relationships. Several methods of analysis, including NOAEL/LOAEL and benchmark dose analyses, are under consideration with regard to the derivation of a new RfC. New data are also available examining the potential carcinogenic effects of toluene. While a single oral study in rats has suggested carcinogenic effects of toluene, limited reporting of the study results confounds interpretation. Adequate inhalation bioassays in rats and mice have not demonstrated a carcinogenic effect of toluene, and the vast majority of genotoxicity evaluations of toluene have been negative. (This document does not necessarily reflect EPA policy.)

512 REFINEMENT OF REFERENCE DOSE (RFD) FOR ZINC.

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USEPA is currently re-evaluating the RfD for zinc. Derivation of an RfD for zinc requires balancing its essentialty functions with its potential adverse effects. Essential trace elements, such as zinc, pose an additional problem of nutrient-nutrient interactions. The excessive intake of one nutrient may interfere with the toxicokinetic properties of another nutrient. Increased zinc intake has been associated with a number of adverse health effects in humans, including decreased erythrocyte Cu-Zn superoxide dismutase (ESOD) activity, hematological effects, decreases in HDL-cholesterol levels, and gastrointestinal effects. Human studies provide evidence that excessive zinc intake may primarily induce copper deficiency. Excessive dietary zinc results in the induction of intestinal metallothionein synthesis. Since metallothionein has a greater binding capacity for copper than for zinc, copper absorbed into the intestinal mucosal cells is sequestered by metallothionein and does not enter the body. Copper deficiency is the primary mechanism for a number of the effects associated with excessive zinc intake, including decreases in HDL-cholesterol levels and ESOD activity. The endpoints associated with copper deficiency appear to be the most sensitive targets of toxicity (occur at doses of Zn as low as 300 ppm). There are limited data to suggest that high zinc intake can decrease iron absorption leading to symptoms of iron deficiency. In deriving an RfD for zinc, these interactions, as well as others, must be considered to determine a level of zinc (4-40 mg/day) that would not result in a disturbance in the balance of other nutrients. (This abstract does not necessarily reflect USEPA policy).

513 RESIDENTIAL SOIL SAMPLING FOR CLEANUP DETERMINATION: COMPARISON OF FIXED-SITE LABORATORY AND FIELD PORTABLE X-RAY FLUORESCENCE (FPXRF) ANALYSIS AND SAMPLE PREPARATION.

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Lead and other metals released from over 100 years of smelting and mining remain a public health concern in Idaho's Silver Valley. In 1986, USEPA and Idaho Department of Environmental Quality started to remove contaminated soil from public areas located near the Bunker Hill Lead Smelter. Recently, USEPA has focused its efforts on sampling and cleanup of lead-laden soils at residences along the South Fork Coeur d'Alene River. Sampling objectives included analysis of soil for lead, arsenic, and zinc for cleanup determination as well as collecting data to test FPXRF as a predictor for fixed-site laboratory analyses (acid digestion/Inductively Coupled Plasma Spectroscopy, AD/ICP) of dried and sieved samples (< 175 micrometer size fraction). FPXRF analyses were performed using a Niton XL 723. Results of FPXRF analyses of 1) dried and sieved samples; 2) dried samples; and 3) unprepared samples (at field moisture) were compared with dried and sieved samples analyzed by AD/ICP. Linear regression models of the dependence of AD/ICP on FPXRF yielded R-square values (R^2) that varied little among sample treatments, although the slopes of the zero intercept regression lines for lead were 0.92, 1.27, and 1.64 for groups 1-3, respectively. For lead, values for R^2 were: 0.94, 0.92, and 0.92 for groups 1-3. For arsenic, the R^2 values were: 0.97, 0.96, and 0.86. For zinc, the R^2 values were: 0.94, 0.86, and 0.94. These results suggest that, for these samples, predictions of AD/ICP from FPXRF measurements would not have been highly affected by drying (moisture content) or sieving (particle size). Future sampling will use FPXRF to analyze unprepared samples with confirmatory AD/ICP analysis of sieved and dried samples when FPXRF results approach soil cleanup levels. Benefits of FPXRF and reduced sample preparation include financial and logistical gains with potential to increase sampling density. Future plans include re-examining sampling and analysis protocols to apply real time data acquisition to adaptive sampling techniques.

514 RISK ASSESSMENT OF METHYLMERCURY INTAKE FROM SPORT FISH TAKEN FROM SELECTED WATER BODIES IN THE NORTHERN SIERRA NEVADA FOOTHILLS, CALIFORNIA.

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The United States Geological Survey collected samples of nine sport fish species from five reservoirs and 14 stream sites in northwestern Sierra Nevada watersheds affected by historical gold mining. Total mercury concentrations were analyzed in composite and individual boneless and skinless fillet samples and reported on a wet weight basis. Mercury concentrations in collected fish species ranged from 0.02 to 1.53 ppm, with the highest concentrations found in spotted bass (mean: 0.85 ppm). In October 2000, the Departments of Environmental Health of Nevada, Placer and Yuba Counties, in cooperation with the Office of Environmental Health Hazard Assessment (OEHHA), issued an interim advisory recommending that sport fishers limit their consumption of several species caught in the study area. OEHHA has since conducted a more detailed assessment to determine human exposure to mercury and the associated potential health risks from consumption of these sport fish. Sample size was sufficient ($\geq$9) for exposure characterization at four species/site combinations. Using the USEPA RfD for methylmercury ($1x10^{-4}$ mg/kg-day), results of the assessment suggest that consumption by pregnant women and young children should be limited for spotted bass from Camp Far West, largemouth bass from Lake Combie, smallmouth bass from Lake Englebright, and channel catfish from Rollins Reservoir. For other sites and/or species, data for similar species from the same water body or the same species from different water bodies were pooled to determine whether consumption should also be limited. Consumption restrictions are also recommended for all other bass species, channel catfish and bluegill from all evaluated reservoirs and brown trout and rainbow trout from the Bear River, South Yuba River, and Deer Creek sites. OEHHA, the agency responsible for issuing state fish advisories, will follow up in developing a final health advisory.

515 EVALUATION OF TEQ EXPOSURE FROM FISH CONSUMPTION RELATIVE TO AVERAGE POPULATION TOTAL EXPOSURE: IMPLICATIONS FOR PCB, PCDF, AND PCDD RISK MANAGEMENT.

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Recognition of a common mode of action for dioxins, dioxin-like PCBs and dibenzofurans has expanded our ability to assess cumulative risk from exposure to these ubiquitous compounds through consideration of TCDD toxic equivalency (TEQ).

PCBs are the second greatest cause of fish advisories, and PCBs are often the greatest contributors to TCDD-TEQ in fish. Because fish consumption is associated with both contaminant risks and health benefits, the incremental risk from this source should be considered within the context of overall TEQ associated risk. In this study, adult TEQ exposure from fish consumption using consumption data and recent fish contaminant data from the literature is explored relative to average population exposure to these agents. Recent estimates presented by EPA suggest fish and seafood contribute just 19% of average dioxin like exposure. However, results from our study indicate that for high level consumers eating from relatively contaminated sites, TCDD-TEQ exposure from fish consumption alone may be 400% greater than the 1 pg TEQ/kg/day average estimated by EPA. This average exposure potentially carries an upper bound cancer risk of one in one thousand. Risk for other groups who consume high levels of fish and seafood from primarily commercial sources is likely less, but is far more uncertain, since data sufficiently sensitive to assess cancer risk from dioxin-like PCBs or even total PCBs, is unavailable for over 96 percent of the seafood they consume. For most adult consumers, however, fish may contribute a small incremental risk from these compounds relative to an unacceptable ambient TCDD-TEQ associated risk, indicating the need for exposure reduction through more holistic environmental efforts rather than simple dietary changes. This proposal was prepared with support of US Department of Energy, under Award No. DE-FG26 00NT40938. However, any opinions, findings, conclusions, or recommendations expressed are the author's and do not necessarily reflect the views of the DOE.

516 RISK ASSESSMENT OF POLYCHLORINATED BIPHENYLS (PCBs) IN INDOOR AIR.

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In the 1970s, residences were built on a site where recent investigation found PCBs (primarily Aroclor 1260) in soil at concentrations as high as 160, 000 mg/kg. Contaminated soil was removed to a target level of 1 mg/kg, but concentrations under the residences were not removed or sampled. Though PCBs are relatively non-volatile, they are complex mixtures and there is a potential for transport of some constituents from soil through the foundation into indoor air. A screening analysis using USEPA's Johnson and Ettinger model for subsurface vapor intrusion estimated greater than 1000 ng/m3 in indoor air, corresponding to a possible cancer risk greater than 1E-5. The modeling was based on a saturation soil concentration of 168 mg/kg. Soil concentrations suggest the presence of non-aqueous phase PCBs in the soil, limiting the utility of the model. Five indoor air samples were collected in three sampling events over four months. Congener-specific analysis using GC/SIM-MS measured total PCBs in indoor air ranging from 112 ng/m3 to 234 ng/m3. The SIM-MS detector has a low detection limit for risk management requirements, but may have resulted in artifacts of sampling, preparation, and analysis. Those potential artifacts required careful analysis of the analytical data to characterize air concentrations. The congeners measured in indoor air were mostly mono-chlorinated. Cancer risk estimates for the detected concentrations range from 1E-6 to 7E-5. Conclusions: 1. The indoor air exposure pathway can be complete for low-volatility chemical mixtures in soil if they contain more volatile components and source concentrations are high. 2. Congener analysis provides information essential to evaluate exposure to PCB vapors, because the congener distribution in air can be very different from that in soil. 3. The Johnson and Ettinger model is useful in identifying potential concerns. 4. Indoor air measurements are technically difficult, but can provide critical information about actual indoor exposures.

517 HUMAN EXPOSURE AND RISK ASSESSMENT OF METHIDATHION IN GREENHOUSES.

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Methidathion (S-2, 3-dihydro-5-methoxy-2-oxo-1, 3, 4-thiadiazol-3-ylmethyl-O, O-dimethyl phosphorodithioate) is a non-systemic, organophosphorous insecticide registered for control of agricultural insect and mite pests on various terrestrial food crops, such as cucumber, citrus and apple in Korea. Human health risk assessment of methidathion was evaluated in greenhouse situation for risk management and for safe re-entry interval establishment. Dermal and inhalation exposure to methidathion were measured when this chemical was mixed/loaded, applied, and during the harvest. And dermal penetration rate was estimated with *in vitro* dermal penetration test using diffusion cell. Oral ADI of methidathion is 0.002mg/kg/day and Oral NOAEL for human is 0.2mg/kg/day. The dermal penetration rate of methidathion was 13.92μg/cm2/hr. The dermal exposure from mixing/loading or application was 2.69mg/hr and 115.6mg/hr, respectively. The average dermal exposure of harvesters for 2days was found to be 1.08mg/hr. There was no significant evidence of inhalation exposure. Hazard Quotient (HQ) and Margin of Exposure (MOE) were used for short-term risk assessment of methidathion. There was no potential risk of mixing/loading, and harvest under the Personal Protective Equipment (PPE) situation

518 ESTIMATES OF ACRYLAMIDE INTAKE FROM THE USE OF PERSONAL CARE PRODUCTS CONTAINING POLYACRYLAMIDE: A MONTE CARLO ANALYSIS.

G. A. Lawrence, C. Van Landingham and A. M. Shipp. *ICF Consulting, Ruston, LA.* Sponsor: M. Friedman.

Estimates of the Lifetime Average Daily Dose (LADD) of acrylamide resulting from the use of representative personal use products containing polyacrylamide have been developed. Because each of the variables that are used to estimate intake can have a range of values and there is uncertainty as to which is the true value, a Monte Carlo analysis was conducted to make maximum use of all of the data. From these data, distributions of values were defined and used to provide a distribution of LADDs for each personal care product. Further, estimates of unit risk factors and doses corresponding to a target risk level, in this case, 1x10-5, were estimated from the bioassay data reported by Johnson et al. (1986) and Friedman et al. (1995) using those approaches typically used by the FDA, the USEPA, and proposed for use by the European Union (EU). Ratios of the estimated acrylamide intake at the median, mean and 90th percentile of the distribution of individual intake values to the lifetime dose corresponding to a target risk level of 1x10-5 were also calculated. All ratios were well below 1, indicating that all were below the target risk level. The conclusion can be drawn that the lifetime cancer risk from the use of polyacrylamide-containing personal care products, in the manner assumed in this Exposure Assessment, are well below acceptable levels. In all cases, even at the 90th percentile on the distribution of LADDs, the ratio of intake to the target dose ranged from 0.01 to 0.000002. Stated differently, use of some of these products and the resulting acrylamide intake from that use would have to increase approximately 100 times at the high end of exposure by more than 500, 000 times to reach the target risk level of 1x10-5. Even if it were assumed that an individual used all of the products together, the estimated intake would still provide a dose that was well below the target risk level.

519 ASSESSING POTENTIAL CANCER RISKS ASSOCIATED WITH INSECTICIDES USED TO CONTROL THE MOSQUITO-BORNE WEST NILE VIRUS: USE OF THE MARGIN OF EXPOSURE APPROACH.

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The 1999 outbreak of the mosquito-borne West Nile Virus in New York City (NYC) resulted in the use of insecticides in an attempt to control mosquitoe populations. Because of the potential for human exposure to insecticides, a human health risk assessment was performed to describe and quantify the potential for health effects, including cancer, due to insecticide spraying. We evaluated cancer risks for six active ingredients in the insecticides, including the organophosphates malathion and naled, the pyrethroids permethrin, resmethrin, and sumithrin, as well as the synergist, piperonyl butoxide. With the exception of naled, there is some evidence that these active ingredients may cause cancer in humans, based on data from animal bioassays. Cancer risks are typically evaluated using a cancer slope factor (CSF). However, CSFs were only available for malathion and permethrin. Of the remaining potentially carcinogenic active ingredients, USEPA specifically recommends using a Margin of Exposure (MOE) approach for evaluating cancer risks for piperonyl butoxide, using the reference dose (RfD) as a point of departure (*i.e.*, a dose associated with a negligible increase in excess cancer). In order to fully evaluate any potential cancer risks in the absence of a CSF or other recommendations, we also used the MOE approach for evaluating cancer risks due to sumithrin and resmethrin, using their RfDs as a point of departure. This poster discusses our rationale for using the MOE approach, and presents the results of our evaluation. For sumithrin and piperonyl butoxide, the MOE is sufficiently high (*i.e.*, the exposure dose was sufficiently lower than the point of departure) as to indicate that cancer risks should be negligible. For resmethrin, the MOE indicates that although there may be some concern for increased cancer risk, excess risks are likely to be very low.

520 RISK ASSESSMENT OF A MILITARY PUBLIC GOLF COURSE SLATED FOR CLOSURE.

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In 1993, Naval Air Station (NAS) Cecil Field was slated for closure by the Base Realignment and Closure Commission and approximately 17, 200 acres is planned for transfer to the private sector. Cecil Field is participating in the Installation

Restoration Program (IRP) under the Department of Defense, to identify, investigate, and cleanup contamination from hazardous materials. Reuse plans have been developed to assist in the property transfer and other closure activities. Anticipated future uses include public buildings and facilities, residential land use, a new runway and industrial use. A risk assessment was performed to evaluate current and future cancer risks and noncancer health hazards from human exposure to a military public golf course potentially contaminated with pesticides, herbicides, insecticides, metals and volatile organic compounds (VOCs). The reasonably maximally exposed (RME) scenarios include an on-unit maintenance worker, future excavation worker, and recreational golfer. Future residential exposures to an adult or child potentially exposed to contaminated soil due to recreational and residential activities were also evaluated. Results from the lifetime cancer risks (RME estimate) were 3E-03 for the future residential adult and 3E-04 for the recreational golfer. For noncancer health effects, the estimated HQ for the residential adult was 24.0 and the estimated HQ for the golfer was 1.5. The cancer risks and non-cancer health hazards for the exposure pathways of the other receptors were much lower. It would be interesting to compare the estimated risks at this previous military golf course to those risks estimated with soil sampling at other private and public golf courses in the southeast.

521 BIODEGRADATION *RULES OF THUMB*: A MEANS TO REDUCE UNCERTAINTY ASSOCIATED WITH PREDICTING HALF-LIVES OF TOXIC, COMBUSTOR-EMITTED CHEMICALS DEPOSITED ONTO SOILS.

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To address the risk presented by combustor-emitted chemicals deposited onto soils, this research examined if general trends, or "*Rules of Thumb*" could be derived that describe the basic influences that soil or chemical characteristics have on biodegradation half-lives. An exhaustive search of the scientific literature was conducted to find documents that provided half-life data and appropriate soil information. Data were extracted from these reports, entered into a database, and statistically analyzed. Soil characteristics examined were pH, organic matter content, soil type, and percent sand, silt and clay. Chemical characteristics examined were formula weight, melting point, water solubility, vapor pressure, Henry's Law constant, Octanol-water partition coefficient (K_{OW}), and soil partition coefficient (K_{OC}). The database provided summary information about the chemicals, the soils in which the chemicals' half-lives were studied, and the range of half-lives observed. Statistical analyses permitted derivation of two *Rules of Thumb*. One *Rule* states that when a chemical is deposited onto a soil, its half-life will be longer in soils high in sand content compared to soils with less sand. The other *Rule* states that a chemical's half-life will be longer in soils with greater amounts of clay compared to soils with less clay. A predictive model was derived that allows estimation of half-lives for chemicals when they are deposited into different soils. The model has the form: Log(half-life)= 2.37×(log[FW]) - 0.145×(log[wat sol]) +0.012×(% sand) +0.753×(log[% clay]) - 8.018 {R^2=0.631, n=110}. Analysis showed, however, that more data are needed to apply this model to a wider range of chemical and soil types. In conclusion, the risk presented by combustor-emitted chemicals that are deposited onto a soil will depend in part on the sand and clay content of that soil. The *Rules of Thumb* capture the important elements of this relationship.

522 EUROPEAN AND USA RBCA, COMPARISON OF HEALTH RISK ASSESSMENT RESULTS FOR OIL REFINERY SITES.

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The potential for residential and industrial redevelopment of a large oil refinery sites were addressed using Tier 2 Risk-Based Corrective Action (RBCA) approach. Two software packages were used in the Tier 2 risk assessments: 1) Van Hall Instituut, Netherlands, RISC-HUMAN, Version 3.1, 1999; and 2) Groundwater Services, Inc.., USA, ASTM RBCA, Version 1.2, 1999. The RISC-HUMAN model is based on methodologies developed by the Netherlands National Institute of Public Health and Environmental Protection (known as RIVM). The GSI model is designed to meet the requirements of the American Society for Testing and Materials (ASTM) PS-104 Standard Provisional Guidance for RBCA. There are several key differences in the models including: 1) the default exposure routes addressed; 2) the methodologies for evaluating carcinogenic risk; 3) the default human exposure and site parameters; and 4) the chemical fate and transport algorithms. Of particular note are the differences in the models for calculating the volatilisation of contaminants from subsurface soil and groundwater to indoor air. A comparison is made between both the model methodologies and results of the risk assessment (i.e., back-calculated risk-based screening levels or RBSLs).

523 THE HEALTH SURVEY OF MUNICIPAL INCINERATOR WORKERS IN JAPAN.

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It is well known that municipal solid waste incinerator (MSWI) fly ash contains heavy metals, dioxins, polyaromatic hydrocarbons, and other organic materials. The workers at MSWIs have a certain possibility to absorb such materials chronically *via* exposure to fly ash. The purpose of this paper is to investigate the health effects of chronic exposure to fly ash on MSWI workers. The subjects were 81 male workers (mean age 42.7) from four MSWIs in the same city. Written informed consents were given from all subjects. Blood and urine samples were corrected in the morning of each study day. Occupational health doctors interviewed each subject about his job history, evaluated the exposure to fly ash, and calculated its total duration. The subjects were classified into four groups; those were Long duration of exposure to fly ash, Short duration of exposure to fly ash, Limited exposure to fly ash, and Control. Blood chemistry, oxidative stress markers, immunological markers, sex related hormones, and neurobehavioral functions were measured and compared among the groups. There were no significant difference or association between measured variables and the groups except oxidative stress markers and neurobehavioral functions. The results of oxidative stress marker are presented elsewhere in this meeting (R Yoshida). The 4 T-scores of POMS (Profile of Mood State), which are Tension-anxiety, Depression-dejection, Fatigue-inertia, and Confusion-bewilderment, increased according to the elongation of the exposure duration. Grip strength of non-dominant side and the ability of short-term memory using a portion of the Symbol Digit Modalities Test increased in Long-exposure-duration group. Although we could not conclude that a certain contaminant of fly ash was responsible to these effects, there may be neuropsychological effects on workers exposed to fly ash.

524 CHARACTERIZING THE HUMAN RISKS FROM MILITARY USE OF NON-LETHAL WEAPONS (NLWS).

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Non-Lethal Weapons (NLWs) are important assets in nontraditional military operations, such as peacekeeping missions, where the use of lethal force may not be a desired first response. Various types of NLWs are considered, including those employing chemical, electromagnetic, mechanical, or acoustic technologies. DoD directives call for these weapons to achieve an appropriate balance between the competing goals of having a low probability of causing death, permanent injury, and collateral materiel damage, and a high probability of having the desired anti-personnel or anti-materiel effects. A conceptual framework for characterizing the risks of human effects from non-lethal weapons has been developed. This framework focuses on the physiological effects caused by the weapons and utilizes tools and techniques of risk assessment, including Dose-Response and exposure information, and decision analysis to provide decision-makers with the probability of intended effects and unintended effects for the exposure scenarios of interest. Preliminary results indicate that force over distance is one unifying metric for some NLWs, and that Dose-Response curves for intended and unintended effects may be estimable for some NLWs, but not others. Field commanders and mission planners could use the resulting risk information to make informed choices with regard to which NLWs would provide the most appropriate combination of target effectiveness and risk for the particular situation or mission. This conceptual framework, and application of it to weapon systems based on various technologies, is a new application of the traditional toxicology and epidemiology based risk assessment approaches. This work was funded by Department of Defense under subcontract PO P66050-DSC0142.

525 RECYCLING WASTES IN AGRICULTURE: EVALUATION OF THE QUÉBEC CRITERIA FOR CADMIUM AND DIOXIN/FURAN BASED ON HUMAN HEALTH ASSESSMENT.

M. O. Fouchécourt and M. Beausoleil. *Institut National de Santé Publique, Montréal, PQ, Canada.* Sponsor: K. Krishnan.

In the province of Québec, Canada, municipal or industrial wastes such as biosolids or calcic amendments can be used as fertilisers in agriculture if they respect several criterias including maximal concentration of 11 inorganic elements and of dioxin/furans (D/F). The objective of this study was (i) to evaluate the exposure of highly exposed individual (HEI) to cadmium (Cd) and D/F when biosolids and calcic amendments (CA) used as a main fertilisers have a concentration of Cd or of D/F equal to the maximal allowed, and (ii) to determine if the risk level is acceptable for the public health. Mean application rates (t d.w./ha-y) of pulp and paper biosolids (1.45 and 2.45 for primary biosolid (B1) and mixed biosolid (B2), respectively) and for CA (0.525) were determined from four scenarios of fertilisation that

respect both agronomic and environmental constraints. Cd and D/F concentrations were set to 3, 10 and 24 mgCd/kg and to 17, 27-50 and 27 ng EQT/kg in B1, B2 and CA, respectively). The contamination of the soil receiving these fertilisers annually was estimated for 100 years. The levels of Cd and D/F in food produced on this soil and on control soils (background concentrations) were estimated by the same method and compared. The HEI was defined as a farmer which spend all its life in a region where soils are mainly fertilised with biosolids and CA, and which is exposed by ingestion (soil, biosolids, CA, contaminated food), inhalation (vapour, dusts) and dermal contact. The method used to estimate the dose of exposure was validated by comparison of the doses estimated from the background concentration in soil with the ones published for Canadians by other authors. Following a long term (100 y), concomitant use of biosolids and CA in the conditions described above, the dose of exposure of the HEI should exceed the reference dose for cadmium (i.e., 0.84 µg/kg-d). The contamination by D/F would lead to a 65% increase of the risk of cancer related to the background level of risk, which is already higher than 1×106

526 HAZARDOUS AIR POLLUTION: A RESEARCH STRATEGY.

M. J. Selgrade. *NHEERL, USEPA, ORD, Research Triangle Park, NC.*

The 1990 Clean Air Act Amendments list 188 chemicals/chemical classes as hazardous air pollutants(HAPs). EPA's implementation of this act includes characterizing residual risks after applying technology-based standards for sources of HAPs, and developing an understanding of risks in urban areas from a subset of 33 HAPs, as well as risks from indoor and mobile sources. Nat'l Air Toxics Assessments (NATA) will monitor progress towards EPA's goal to eliminate unacceptable risks of health problems from HAPs. The 1st NATA reveals many uncertainties. A research strategy is needed to reduce the uncertainties in risk assessment that result from lack of information. The research needed differs considerably from that needed to address the 6 criteria pollutants. The sheer number of chemicals is daunting. Thus, it is necessary to focus attention on those HAPs which pose the greatest risk to humans, and if possible group HAPs based on physicochemical properties, structure/activity, and mechanisms of toxicity, in order to increase the usefulness of information gathered on any one HAP. HAPs can pollute different media; hence, inhalation may not be the only or even primary exposure route. Research is therefore needed to consider the fate & transport of HAPs once they leave the source, to characterize personal exposures, and to consider methods for dealing with aggregate risks of exposure of multiple organ systems by more than one route. Because much of the current data come from studies using other than inhalation exposures, pharmacokinetic models are needed to facilitate route to route extrapolation. Information is also needed to deal with cumulative risk of exposures to HAP mixtures and to consider the risks of sensitive populations. Unlike the criteria pollutants, HAPs risk assessments will have to rely more heavily on animal to human extrapolation. The purpose of this symposium is to propose a multidisciplinary approach for meeting HAP research needs, provide some examples, and encourage the researchers to think outside the traditional box of one chemical, one route of exposure, one species studies. This abstract does not reflect EPA policy

527 EXPOSURE ASSESSMENT TO HAZARDOUS AIR POLLUTANTS.

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When Hazardous Air Pollutants (HAPs) are prioritized according to potential risk, it is critical to consider total exposure from both ambient and non-ambient emissions. Exposure to HAPs from non-ambient sources can exceed that from ambient emissions. Small sources of HAPs in close proximity to people (releases within homes and use of personal products) can dominate the inhalation exposure and HAPs have other exposure routes (dermal/ingestion). For example, inhalation exposure to formaldehyde indoors with new furnishings, ingestion exposure to PAH from charcoal cooked foods, and ingestion and dermal exposure to dust containing lead and pesticides for toddlers can exceed inhalation exposures from ambient air sources. Characterization of HAPs by source type and physiochemical properties is important because not all HAPs can be individually determined in a cost effective manner. The contribution of ambient air emissions to indoor air is a function of environmental transport and penetration factors into homes. The indoor air concentration is critical to determine since people spend the majority of their time indoors or in transit. We have measured indoor/outdoor and personal/outdoor air ratios that were greatly in excess of one for many of the HAPs, such as the VOCs, tetrachloroethyene, chloroform and 1, 4 dichlorobenzene and the aldehydes, formaldehyde and acetylaldehyde. The ratio for particulate matter mass was less than one but, individual PAHs and metals exceeded unity. Differences in exposure to HAPs across the population due to age (child vs adult), gender, socioeconomic and cultural differences exist as these factors affect the time spent in different locations and activities that alter the multi-route exposure to the HAPs. Inhalation exposure measurements are typically integrated averages of ½ to two days, but acute exposures can exist that may be of toxiciological importance. Thus, reported ambient HAP concentrations are not good surrogates for exposure, and we need to determine which HAP exposures are dominated by ambient emissions and what time scale are important for those exposures.

528 STRUCTURE-ACTIVITY APPROACHES AND DATA EXPLORATION TOOLS FOR PRIORITIZING AND ASSESSING THE TOXICITY OF HAZARDOUS AIR POLLUTANTS.

A. M. Richard and C. R. Williams. *NHEERL, USEPA, Research Triangle Park, NC.* Sponsor: M. Selgrade.

Hazardous Air Pollutants (HAPs) refers to a set of structurally diverse environmental chemicals, many with limited toxicity data, that have been grouped, not due to chemical or biological similarity, but rather due to a common route of exposure (inhalation), environmental media (air) and regulatory framework. Hence, this set of chemicals provides particular challenges to structure-activity relationship (SAR) and data mining approaches. These approaches can be productively applied to characterization and testing prioritization of HAPs chemicals, but analyses must reach beyond this set of chemicals to construct larger chemical groupings according to either common structures and mechanisms of action, or a common endpoint of concern. Commercial toxicity prediction programs can apply algorithms derived from large sets of chemicals to estimating general toxicity properties of the HAPs chemicals. Another type of SAR approach, more focused and exploratory in nature, has successfully characterized structural classifiers of activity and determinants of potency among a set of diverse volatile organics, many HAPs included, that have been evaluated for their ability to produce rat nasal histopathology. The electronic and reactivity indices identified have provided a basis for exploring associations with the sub-chronic nasal irritation endpoint for which larger data sets are available. Finally, a new initiative to enable structure-based searching and exploration of public toxicity data bases will be reported that has the potential to greatly enhance current capabilities to gather relevant data with respect to HAPs chemicals. Such tools will facilitate analogue identification and hypothesis generation with respect to characterization of HAPs spanning multiple classes and toxicity endpoints of concern. This abstract does not reflect EPA policy.

529 CONCENTRATION - DURATION RELATIONSHIPS FOR NON-CANCER HEALTH EFFECTS OF EXPOSURE TO HAZARDOUS AIR POLLUTANTS.

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EPA is charged with assessing the risks of both acute and chronic exposures to hazardous air pollutants (HAPs). The emissions from sources of HAPs are often characterized as temporally-averaged values, however, patterns of exposure not captured in such measures may influence non-cancer toxicity. This research program examined non-cancer health effects as a function of temporal exposure patterns, with the goal of improving models to predict toxicity across exposure scenarios. Currently, temporal adjustments in risk assessment typically are based on Haber's rule ($C \times t = K$) or ($C^n \times t = K$), where C represent concentration, t time (duration) of exposure, K a constant toxic effect and n, when used, is empirically derived. Several general conclusions were derived from this work. Concentration is more important than time: where C and t were manipulated the influence of concentration predominated over time in contrast to the equal influence of C and t in the linear form of Haber's rule. Target tissue dose. Understanding the relationship between exposure and target tissue dose was better at predicting toxicity than was Haber's rule. Target tissue sensitivity changes over time: variations in target tissue sensitivity are not accounted for in standard $C \times t$ calculations. Interpretation of standard toxicity assays: standard toxicity assays do not address acute exposures during critical windows of sensitivity. Quantitative models: models with parameters reflecting physiological entities improved extrapolation across species and age as well as exposure concentration and duration. Understanding Mechanisms: having mechanistic information improved predictions across exposure conditions. These results demonstrate the importance of considering temporal exposure variations when predicting the risks of adverse non-cancer health outcomes. This abstract does not reflect EPA policy.

530 MODEL-BASED APPROACHES TO DOSE EXTRAPOLATION.

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The preponderance of non-inhalation route toxicological evaluations of volatile chemicals places a premium on methodologies for conducting route-to-route dose extrapolations. Such methodologies are increasingly important for industry and regulatory agencies seeking to conduct risk assessments of chemicals that pose a hazard predominantly by the inhalation route, because they facilitate the use of data from alternative dose-routes. For these compounds, a reliable capacity to perform route-to-route extrapolation increases the database for conducting risk assessments based on target tissue dosimetry. Physiologically-based pharmacokinetic (PBPK) models with route specific components link dose-routes through tissue dosimetry, and are the leading tool for conducting dose-route extrapolations. Model-based approaches to estimate dose-equivalence (in terms of mg per kg per time) for various exposure routes, such as inhalation, dermal, oral, gavage, and intraperitoneal have been computed for a few hazardous air pollutants (ex. vinyl acetate and styrene). Minimum data needs to perform adequate route specific dosimetry, and their associated advantages and limitations, have been characterized. Such models can also be used for mode-of-action hypotheses testing by correlating multiple internal dose metrics to the observed response data.

531 AIR TOXICS RESEARCH NEEDS IN THE 21ST CENTURY, SUMMARY.

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The 1990 Clean Air Act Amendments (CAAA) listed 188 chemicals and chemical classes as hazardous air pollutants (HAP). In most cases the health effect of concern for HAP exposure is cancer risk, while a limited list of HAPs are known or suspected to cause or exacerbate other diseases (e.g., asthma, fibrosis, neurodegenerative diseases, developmental effects). The complexity of working with this extensive list of HAPs arises not only from the exhaustive list, but also the certainty that exposures will be to mixtures. This is further complicated by the limited information on exposure levels as well as health effects of individual compounds, nevertheless the effects of mixtures (additive, synergistic, antagonistic). Selection of representative classes of HAPs may be useful, but will not resolve all of the issues and in the case of disease causation assumes that all of the potential mechanisms are identical. Furthermore, identification of sensitive and susceptible populations remains incomplete. The potential matrix becomes overwhelming and tactics need to be developed to focus on the most likely scenarios of exposure. One approach is to assess actual exposure that is most representative of a substantial fraction of the population and generate an appropriate mixture. Issues to review are the limitations of exposure assessment, the applicability of the resulting mixture, and the approaches that could be utilized having this information. Alternatively, in the absence of portions of this data set what would be the most scientifically plausible approach to filling the data gaps. This information will be critical to advance the study of health effects to real world exposure to HAPs.

532 TOXICOLOGICAL APPLICATIONS OF METABONOMICS TECHNOLOGY.

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Toxicants, by definition, disrupt the normal composition and flux of endogenous biochemicals in, or through, key intermediary cellular metabolic pathways. These disruptions, either directly or indirectly, alter the blood that percolates through the target tissues. The diagnostic utility of any one trace biomolecule is limited by the number of variables affecting its concentration *in situ* and by the commonality of biochemical processes disrupted by toxicants. However, if a significant number of trace molecules are monitored, the overall pattern, or " fingerprint" produced may be more consistent and predictive than any one marker. Obtaining such comprehensive biochemical information is made possible using high-field nuclear magnetic resonance (NMR) spectroscopy coupled with pattern recognition technology. Urine and serum are routinely studied, but virtually any biofluid can be used for metabonomic analysis. Magic-angle spinning NMR technology enables similar information to be garnered from tissues as well. The power of the technology lies in its ability to non-invasively assess animal physiology thus allowing temporal evaluation of the metabolic consequences of toxicity. Onset and regression of toxicity can be determined within a single animal, target organs identified, mechanistic information obtained and biomarkers of toxicity established. When coupled with genomic and proteomic technologies, metabonomics permits complete assessment of toxicity from genotype through phenotype. The objectives of this symposium are to introduce the principles of metabonomics, describe its application emphasizing both the strengths and weaknesses and to present a vision of the future for this emerging technology.

533 METABONOMIC APPROACHES TO UNDERSTANDING THE CONSEQUENCES OF DRUG TOXICITY AND GENE FUNCTION.

J. K. Nicholson. *Biological Chemistry, Faculty of Medicine, Imperial College, University of London, London, United Kingdom.* Sponsor: D. Robertson.

High frequency ^{1}H NMR spectroscopy provides a rapid method of characterizing and quantifying a wide range of metabolites in untreated biological fluids and tissues and a powerful means of exploring the biochemical effects of drug-induced toxicological processes. ^{1}H NMR spectra of biofluids are highly complex, containing signals from thousands of metabolites representing many key biochemical pathways. The ^{1}H NMR-generated biofluid metabolite profiles are characteristically changed in different toxicological conditions according to the exact site and mechanism of the lesion. Thus interrogation of the biofluid NMR data can give direct diagnostic information and aid the detection of novel biomarkers of toxic effect. Recent advances in MAS-NMR spectroscopy also allow high resolution ^{1}H data to be collected on small intact tissue samples, thus giving metabolic information that is complementary to those obtained from biofluid NMR. By use of computer pattern recognition (PR) methods, complex biofluid/tissue NMR data can be reduced and analyzed quantitatively to provide PR maps showing the physiological and toxicological effects of test xenobiotics in pre-development drug toxicity studies and the effects of genetic modification. Neural network, rule induction and other expert system approaches can also be implemented to give direct diagnostic outputs on toxicity type based on NMR input data. NMR-PR based diagnostics can be extremely sensitive for the detection of low level damage in a variety of organ systems and is potentially a powerful new adjunct to conventional toxicological procedures for lead compound selection. With the advent of flow injection NMR technology, it is now possible to analyze hundreds of biofluid samples per day using one NMR spectrometer and to use NMR as a novel tool for toxicological screening. The NMR-PR and expert system approach to rapid *in vivo* toxicological assessment of drugs will be illustrated for a wide range of drug and toxin-induced lesions in both experimental animals and man.

534 APPLICATION OF METABONOMIC EXPERT SYSTEMS TO THE STUDY OF PHYSIOLOGICAL AND TOXICOLOGICAL VARIATION.

E. Holmes. *Biological Chemistry, ICMS London University, South Kensington, London, United Kingdom.* Sponsor: D. Robertson.

One of the major applications of NMR-based metabonomic technology in the pharmaceutical industry involves advanced chemometric analysis of NMR spectra of biological matrices with a view to establishing and predicting the pharmacological and pathophysiological consequences of drug candidates. Using such technology, overt toxic insults are easily detected as perturbations in the 1H NMR spectral profiles and expert systems can be constructed for efficient classification and prediction of drug toxicities. However, many factors can be responsible for generating biochemical alterations in the baseline urinary profile including change in diet, diurnal variation, hormonal cycles, changes in temperature, poor health, level of activity, genetic drift and bacterial contamination of urine, and all of these factors may have implications on the toxicologic response of an animal. NMR-based metabonomic methods can be used to establish the degree and precise nature of normal physiological variation for a population of animals or humans. Moreover, mathematical data filtering methods can be used to deconvolve confounding physiological effects, thereby removing sources of variation that are unrelated to the class of toxicity. Sophisticated expert systems that can compensate for physiological, pharmacological and pathophysiological variation can thus be established.

535 METABONOMIC AND GENOMIC INVESTIGATION OF RENAL PAPILLARY NECROSIS (RPN).

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Metabonomics and oligonucleotide-based microarray technology were used in a comparative study with compound 393277, an experimental agent inducing RPN. Bromoethanamine (BEA) was used as a positive control. Papillary injury was evident microscopically at 4 h postdose for 393277 and 24 h for BEA. NMR showed

that 393277 treatment induced changes previously identified as typical for papillary toxicants including altered excretion of trimethylamine-N-oxide and decreases in 2-oxoglutarate and citrate. Trajectory analysis of NMR data indicated a return to 'normal' by 72 h following either single or repeat administration. In several 393277-treated animals, elevated taurine combined with lower TCA cycle intermediates suggested hepatic injury. Histologic examination revealed hepatocellular vacuolation at 48 h that resolved by 96 h. Gene expression changes in liver and kidney papilla were also maximal at 48 h with few changes persisting at 96 h. In the papilla, expression changes were noted for genes common to both compounds including immediate early genes (c-fos, jun), general stress genes (MT, HO), and inflammatory markers (gro, MIP-2). Corresponding to the observed hepatotoxicity, 393277 altered expression of more genes in the liver than did BEA. Genes associated with beta-oxidation and fatty acid metabolism were affected in both liver and kidney, consistent with the NMR data. In the ovary of 393277, but not BEA-treated animals, a marked reduction in expression of genes associated with estradiol and progesterone was observed. This finding agrees with the pathology and with data demonstrating estral cycle disruption associated with decreased levels of circulating hormones. This study documents that both metabonomics and genomics have the ability to predict target organ toxicity following acute dosing and that parallels can be drawn between the technologies.

536 METABONOMIC TECHNOLOGY AND THE PHARMACEUTICAL INDUSTRY: THE COMET CONSORTIUM.

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Metabonomics technology has the potential to impact drug-development from early in the discovery process through clinical trials. The non-invasive assessment of toxicity (or efficacy) enables rapid-throughput screening while the potential for serial assessment of toxicity within a single animal minimizes drug requirements. Beyond screening, early identification of biomarkers of either efficacy or toxicity is engendered. This identification of biomarkers is more than just an alternative to traditional clinical chemistry measurements. Metabonomics enables identification of biomarkers where currently none exist. The non-invasive nature of the technique makes for ready export to the clinic. The spectrum of changes and the ease of identifying temporal responses allow for novel mechanistic information to be obtained adding significantly to the assessment of safety. The value of pattern recognition techniques to toxicological endpoints is realized only if a valid pattern is established. The role of the consortium on metabonomics (COMET) is to establish a comprehensive database that defines the chemical space encompassed by hepatotoxins and nephrotoxins. The consortium is a research endeavor of 6 pharmaceutical companies collaborating under the auspices of Imperial College in London. This presentation will provide practical examples of the application of metabonomic technology within the pharmaceutical industry. 'Lessons Learned' from the COMET consortium will be presented and cross-company validation efforts described.

537 TRANSPLACENTAL CARCINOGENESIS OF ENVIRONMENTAL TOXICANTS.

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A growing body of scientific evidence has shown that the developing fetus and neonate exhibit different sensitivities and responses to environmental chemicals than do adult organisms, and that exposure of parents before conception can alter the risk of cancer and other diseases in the offspring. Developmental and stage specific effects are often seen as a result of the dynamic nature of the developing organism, which presents a constantly changing biochemical picture as various enzyme and signal transduction systems are turned on or off during growth and maturation. The higher sensitivity of the fetus to certain carcinogens has long term implications for the regulation of potentially toxic chemicals. Several studies have suggested that the process of cancer initiation may begin *in utero* following exposure of the fetus to carcinogenic agents. Thus, the fetus and neonate are not "little adults", and further understanding of the toxic effects of chemicals at both the biochemical and molecular/genetic level are needed in order to make rational regulatory decisions, to devise chemopreventive strategies for exposed populations, and to elucidate the unique characteristics of tumors resulting from damage initiated during the early developmental stages. The first speaker will discuss recent data showing that pre-conceptional exposure of male mice to carcinogens can lead to epigenetic changes in hormones that could influence tumor development in subsequent generations. The second speaker will review alterations that occur at oncogenic loci following *in utero* exposure of mice to 3-methylcholanthrene, contrasting the differences between adult and fetal exposures. The third speaker will continue on this theme and describe the role of the *N*-acetyltransferases in determining the genotoxicity of aromatic amines in the fetus and neonate. The last speaker will demonstrate the effects of tobacco smoke exposure on 4-aminobiphenyl-hemoglobin adduct formation and mutation at the HPRT locus in human maternal and fetal lymphocytes to highlight the effects of *in utero* exposures on the fetus in the human population.

538 EPIGENETIC ASPECTS OF PERINATAL CARCINOGENESIS: HORMONAL MODULATION.

L. M. Anderson. *Laboratory of Comparative Carcinogenesis, NCI, Frederick, MD.*

Epidemiological evidence continues to implicate early life exposures as factors in cancer risk, and certain forms of childhood cancer, including leukemia, appear to be increasing. Preconceptional exposures of fathers, occupationally to chemicals such as metals, solvents, and pesticides, and to tobacco smoke, have been linked to increased risk of childhood cancers. Preconceptional carcinogenesis after exposures of fathers has been clearly demonstrated in animal models, for most categories of chemical carcinogens and for radiation. The phenomenon in animals suggests that a novel mechanism may be in play, since the incidence of effect is higher by several orders of magnitude than the rate of any known specific locus mutation, and the inheritance pattern may be non-Mendelian. Since the tumors that are increased after preconceptional exposure of animals are most often those that have a significant spontaneous incidence, we postulated that the mechanism could be epigenetic and involve changes in hormones that influence tumor development. We have pursued this hypothesis using chromium(III) as the preconceptional exposure agent. Cr(III) is a common human occupational exposure metal and a demonstrated preconceptional carcinogen in mice. After exposure of male Swiss mice to this chemical, their offspring at 10 weeks of age have been found to have marked, highly significant alterations in average levels of serum corticosterone, glucose, and insulin-like growth factor 1, compared with offspring of vehicle-treated fathers. Since all of these factors have known potential to influence tumor growth, these findings confirm the hypothesis. To determine whether the hormone alterations relate in fact to tissue changes, we have utilized microarray analysis, starting with liver. The changes in corticosterone correlated strongly with gene expression for insulin-like growth factor binding protein 1, a regulatory protein for IGF1. Such transgenerational effects on hormones in offspring may have wide implications.

539 ALTERATIONS IN ONCOGENES AND TUMOR SUPPRESSOR GENES IN TRANSPLACENTALLY-INDUCED TUMORS.

M. S. Miller and M. C. Mizesko. *Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC.*

Fetal mice exhibit differential sensitivity to carcinogens that is determined by their ability to metabolize xenotoxicants. Following *in utero* exposure to 3-methylcholanthrene (MC), the treated offspring demonstrated increased sensitivity for tumor formation compared to exposed adults. [D2 x B6D2F1]F2 and Balb/c mice develop lung tumors 6-12 months after birth, with the F2 mice also developing liver tumors. The tumor incidence correlated with expression and inducibility of *Cyp1a1*. Conversely, fetal mice were resistant to tumor induction by 2-amino-3-methylimidazo[4, 5-*f*]quinoline (IQ), probably due to the lack of *Cyp1a2* expression during gestation. MC-induced tumors from the [D2 x B6D2F1]F2 and Balb/c mice demonstrated a high incidence of mutations in Ki-*ras*, with 79% and 56% of the lung lesions exhibiting mutations in exon 1, respectively. [D2 x B6D2F1]F2 mice showed predominantly G→T transversions (84%) whereas the Balb/c mice had mostly G→C mutations (62%). In both studies, the type of Ki-*ras* mutation was associated with the histological stage of the tumors. Though no alterations were found in the *p53* gene, alterations in the *Rb/Ink4a* axis were seen. All 14 of the larger lung tumors examined from [D2 x B6D2F1]F2 mice displayed decreased levels of *Rb* RNA and protein. A low incidence of alterations at the *Ink4a* locus in both lung (15%) and liver (27%) tumors was seen. However, whereas adult lung lesions exhibited mainly promoter hypermethylation and a low incidence of homozygous deletions, mice exposed *in utero* had low incidences of both point mutations and hypermethylation. The type of alteration at the Ki-*ras* locus appeared to influence the type of damage that occurred in *Ink4a*, suggesting that the type of mutation induced in Ki-*ras* may influence tumor progression. Thus, the developing organism is more sensitive to certain carcinogens and exhibits a distinctive tumor pathogenesis relative to adults.

540 PRE- AND POSTNATAL EXPRESSION OF N-ACETYLTRANSFERASES (NAT): POTENTIAL ROLE IN AROMATIC AMINE GENOTOXICITY.

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Children and adults often respond differently to xenobiotics. This may be due, at least in part, to alterations in the expression of biotransformation enzymes that can affect the formation of reactive and/or non-toxic products. Studies in mice have fo-

cused on NAT2, an enzyme that may be involved in regulation of folate metabolism and play a role in neural tube development. NAT2 has been detected in murine pre-implantation embryos with the NAT2 protein and associated capacity for acetylation increasing from birth to sexual maturity. Since activation of aromatic amine carcinogens involves reactions catalyzed by NAT1 and NAT2, the developmental expression of both genes is of interest. In C57Bl/6J mice, NAT1 and NAT2 mRNAs were detected at gestational day (GD) 10, the earliest time evaluated, with fetal *N*-acetylation of 4-aminobiphenyl (4ABP) measured at GD 10, 15 and 18. After birth, hepatic expression of NAT1 and NAT2, determined by quantitative RT-PCR, continued to increase with age. This trend was also observed when NAT activities were assessed. Hepatic *N*-acetylation of *p*-aminobenzoic acid, a murine NAT2 selective substrate, isoniazid, a murine NAT1 selective substrate, as well as the carcinogenic aromatic amines 2-aminofluorene and 4ABP, substrates for both isoforms, increased with increasing age. *in utero* exposure to 4ABP resulted in fetal 4ABP-DNA adducts, reflecting maternal and possibly fetal activation of 4ABP. Neonatal exposure to 4ABP resulted in levels of hepatic 4ABP-DNA adducts that were significantly lower at neonatal day 4 than in adult liver. Thus, the capacity to acetylate aromatic amine carcinogens is present pre- and postnatally. While fetal and maternal biotransformation contribute to 4ABP genotoxicity during gestation, there is sufficient neonatal biotransformation to result in 4ABP-DNA adducts. The activation of aromatic amine carcinogens and the formation of DNA adducts during development may result in critical mutations that increase risk of childhood or adult cancer. (Supported by ES 09812 and ES 10047).

541 APPLICATION AND USE OF HEMOGLOBIN AS A BIOMARKER OF TOBACCO SMOKE EXPOSURE.

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Smoking during pregnancy has been linked to a variety of adverse outcomes, including low birthweight, spontaneous abortion, and infant death. Low birthweight shows the clearest and most consistent association with maternal smoking. Evidence suggests that a Dose-Response relationship exists between cigarette consumption, especially during the third trimester, and birth weight. Women who smoke during pregnancy expose themselves, as well as the fetus, to numerous carcinogens, many of which may be harmful to the fetus. Although much data concerning the effects of smoking is known, few studies have investigated the effect of tobacco smoke on the developing fetus. Three of the most widely studied carcinogens in tobacco smoke include the potent bladder carcinogen 4-aminobiphenyl, as well as the carcinogens benzo(a)pyrene and the tobacco specific nitrosamines. Numerous biological markers of exposure to tobacco smoke carcinogens have been proposed and investigated. Recently, the utilization of hemoglobin (Hb) adducts of tobacco smoke compounds have emerged as a viable biomarkers of tobacco smoke carcinogen exposure. Hb adducts will accumulate in the circulation, allowing their use as biomonitors of exposure to various agents, such as those carcinogens found in tobacco smoke. In this presentation, we will discuss a series of studies that have helped define the use of Hb as a biological marker to assess exposure to carcinogens. In addition, we will discuss the following hypotheses related to the use of Hb adducts as biomarkers of exposure assessment. These include (1) that the formation of fetal Hb tobacco smoke carcinogen adducts is proportional to maternal smoking as well as maternal Hb adducts; (2) that the formation of maternal and fetal Hb adducts are dependent on maternal and fetal genotype to glutathione S-transferase M1 (GSTM1), glutathione S-transferase T1 (GSTT1), *N*-acetyltransferases (NAT1 and NAT2), or cytochrome CYP1A1; and (3) that there is a correlation between the formation of DNA adducts and maternal and fetal Hb adducts.

542 INTRODUCTION TO PERFLUOROOCTANE SULFONATE (PFOS).

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Perfluorooctanesulfonate (PFOS) is a perfluorinated organic acid that has been used as a surfactant in products such as fire fighting foams. It is also formed by the environmental or metabolic degradation of other fluorochemical products used on items such as carpets, clothing, and food containers. PFOS does not appear to be further degraded by humans or environmental processes. Analytical techniques in the 1960s could identify organic fluorine in human serum at levels ~ 0.10 ppm. In the 1990s, improved methods and instrumentation allowed routine measurements of specific organofluorine chemicals in human and environmental samples at levels of ~ 0.01 ppm. PFOS has been measured at low concentrations in serum samples from human populations, in various species of wildlife, in surface waters and in other environmental media. Occupational workers have measured serum PFOS levels that averaged 2.0 ppm with highest levels at ~11 ppm. In non-occupational human samples, the upper 95th percentile serum concentration is approximately 0.1 ppm. The halflife of PFOS in the serum of retired workers is currently estimated to be several years. Environmental matrices are being examined to understand the dispersion of PFOS and other fluorochemicals in the food chain, general environment and in the vicinity of manufacturing and heavy use. Results of such studies indicate that PFOS is widely dispersed in the environment. Substantial information has been developed and continues to be developed on the possible biological effects of exposures. The mammalian toxicology database has grown rapidly. Also, several epidemiological investigations in exposed workers have been completed. Numerous acute and chronic toxicity studies involving freshwater and marine organisms also have been conducted. The aggregate data, to be summarized at this workshop, can permit a basic understanding of exposure, effects, mechanism and risk to the environment, wildlife and humans.

543 UNDERSTANDING THE GLOBAL DISTRIBUTION AND ENVIRONMENTAL EFFECTS OF PFOS.

W. J. Warren-Hicks. *The Cadmus Group, Durham, NC.* Sponsor: J. Moore.

Perfluorooctane sulfonic acid (PFOS) and its salts are fully fluorinated organic molecules produced synthetically by electrochemical fluorination or from the degradation of other fluorochemical products produced by electrochemical fluorination. As a fully fluorinated organic molecule, PFOS is very stable and resists further degradation. Substantial information related to human and environmental exposures to PFOS has been developed providing evidence of widespread distribution in humans and the environment. PFOS at very low levels has been identified in serum and tissue samples from both occupationally and non-occupationally exposed human populations, in various species of wildlife, and in surface waters and other environmental media. An extensive database has been, and continues to be, developed on the possible biological effects of these exposures. Based on the existing body of information presented here, the observed levels of PFOS from a wide variety of samples have not been associated with identifiable adverse effects on human health, wildlife, or the environment. Additional data currently under development from ongoing studies will be used in the future to refine this initial assessment. This presentation presents a comprehensive ecological risk assessment of PFOS using existing exposure and effects data. Analytical methods for the determination of exposure concentrations and toxicology of PFOS are presented. The analyses suggest that levels found in the environment and in wildlife are not associated with adverse effects. Additional research is now underway that will be used to refine this initial assessment.

544 THE HEALTH EFFECTS OF PFOS.

J. Seed. *OPPT, USEPA, Washington, DC.* Sponsor: J. Moore.

Numerous repeat-dose oral toxicity studies on PFOS have been conducted in rats and primates. In rat studies, toxicity has been observed at doses of 2 mg/kg/day and above and effects include increases in liver enzymes, hepatic vacuolization and hepatocellular hypertrophy, gastrointestinal effects, weight loss, convulsions, and death. In rhesus monkey studies, toxicity has been observed at doses of 1.5 mg/kg/day and above, and effects include anorexia, emesis, diarrhea, hypoactivity, prostration, convulsions, atrophy of the salivary glands and the pancreas, marked decreases in serum cholesterol, and lipid depletion in the adrenals, and death. At doses as low as 0.75 mg/kg/day, cynomolgus monkeys exhibited low food consumption, excessive salivation, labored breathing, hypoactivity, ataxia, hepatic vacuolization and hepatocellular hypertrophy, significant reductions in serum cholesterol levels, and death. Developmental toxicity was evident at doses of 5 mg/kg/day and above in rats administered PFOS during gestation, and at doses of 2.5 mg/kg/day and above in rabbits. In a two-generation reproductive toxicity study in rats, all F1 pups in the 3.2 mg/kg/day dose group died within a day after birth while close to 30% of the F1 pups in the 1.6 mg/kg/day dose group died within 4 days after birth. The NOAEL and LOAEL for the F2 pups were 0.1 mg/kg/day and 0.4 mg/kg/day, respectively, based on reductions in pup body weight. A subsequent cross fostering study showed that the perinatal death was due to *in utero* exposure. In addition, reductions in serum lipids and cholesterol synthesis do not appear to play a significant role in the perinatal mortality. A report on the results of a two-year feeding study in Cr1:Cd (SD) rats is expected in late 2001. This abstract does not reflect EPA policy.

545 MOLECULAR MECHANISMS OF PERFLUOROOCTANESULFONATE TOXICITY.

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The evidence regarding perfluorooctanesulfonate (PFOS) toxicity is strongly suggestive of a primary metabolic disorder. Early indicators of exposure to PFOS in rats include cachexia, hepatomegaly that results from expansion of the mitochondrial, endoplasmic reticulum and peroxisomal bodies, decreased serum glucose,

triglyceride and cholesterol, and the accumulation of lipid vacuoles in liver tissue. In view of the fact that PFOS is a perfluorinated fatty acid, we questioned whether its toxicity might owe to competing reactions for fatty acid metabolism in the cell. Although PFOS and its metabolic precursor perfluorooctanesulfonamides (PFOSN) do not inhibit mitochondrial fatty acid oxidation, they do interfere with mitochondrial bioenergetics. PFOS causes a nonspecific increase in permeability of mitochondrial membranes, whereas the primary and secondary sulfonamides are highly specific and extremely potent uncouplers of oxidative phosphorylation. PFOS and its sulfonamides also interfere with fatty acid metabolism in the cell by activating peroxisome proliferator activated receptor to stimulate the proliferation of hepatic peroxisomes and enhanced expression of peroxisomal enzyme activities in rats *in vivo* and in cell culture. Guinea pigs, however, are unresponsive to PFOS or PFOSN-induced peroxisome proliferation. PFOS exposure also causes a dramatic stimulation of CYP4A1 expression in rats, but not guinea pigs. In conclusion, PFOS and its sulfonamide precursors exert multiple effects to interfere with intermediary metabolism by the cell, indicating that it is quite probable that metabolic dysfunction is the basis for the generalized wasting syndrome and perinatal mortality that characterize PFOS and PFOSN toxicity in rats. However, there exists a discerning species-related difference in susceptibility to metabolic dysfunction, which raises some uncertainty whether rats provide a reliable indicator of potential adverse outcomes associated with human exposures to PFOS or its precursor sulfonamides. (This work was supported by The 3M Company.)

546 CHALLENGES OF RISK CHARACTERIZATION FOR PERFLUOROOCTANESULFONATE (PFOS): A PERSISTENT, PERVASIVE FLUOROCHEMICAL.

G. S. Omenn. *University of Michigan, Ann Arbor, MI.* Sponsor: J. Moore.

In traditional assessments used in risk characterization, external dose is generally measured or estimated in the absence of direct measurement of biomarkers of exposure in serum or other tissues. Absorption, distribution, metabolism, and excretion differences across species are often unknown; thus, generic uncertainty factors, not chemical-specific in nature, are commonly used. Such approaches can yield estimates of exposure and risk that are so widely divergent as to undermine the confidence of the scientific community. Scientific data for perfluorooctanesulfonate (PFOS) present characteristics that may permit more refined assessment approaches. PFOS has been identified in non-occupationally exposed human population serum samples at low parts per billion levels. The origin of PFOS in human sera is not clearly understood; most likely, it comes from a combination of environmental and product exposures. Because PFOS is persistent, serum levels integrate exposure over time from all sources. Furthermore, PFOS serum concentration has been shown to approximate liver tissue concentration in humans and in laboratory animals at levels observed in human populations. Using such knowledge can reduce some of the uncertainty inherent in the typical approach to risk assessment. The approach we recommend for PFOS involves comparisons of the range of serum levels documented in both epidemiological and toxicological studies of exposed populations. The presentation will examine these assumptions and discuss limitations related to the nature and extent of human exposure data, the assumption that PFOS is the sole or major toxicant, and the likelihood of genetic variation in biotransformation and in susceptibility to adverse effects.

547 EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) AND HYPOXIA ON CARDIAC VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXPRESSION.

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A myocardial oxygen gradient during embryo development is thought to stimulate coronary angiogenesis and VEGF is one factor that regulates this process. Previously, we have demonstrated that TCDD inhibits the branching of coronary arteries in the developing chick embryo heart. Thus, we tested the hypothesis that hypoxia can regulate cardiac VEGF mRNA expression and TCDD can reduce cardiac VEGF mRNA expression. To determined if hypoxic incubation conditions induced the cardiac hypoxia, chicken eggs were injected with 0.9% saline (control) or ^{3}H-misonidazole (MISO, a tissue hypoxia marker) in saline on incubation days 7 or 9 (D7 or D9) and the incubated for 24 hr under 20% or 15% O_2. Misonidazole is reduced metabolically to a reactive intermediate when tissue oxygen levels are low and will bind to cellular macromolecules. Hearts were collected on D8 and D10 and analyzed for MISO binding by scintillation counting. Hypoxia induced a 155 ± 14% and 3300 ± 60% increase in MISO binding on D8 and D10, respectively. To determined if these same hypoxic incubation conditions induced cardiac VEGF mRNA, chicken eggs on D7 and D9 were incubated for 24 hr under 20% or 15% O_2. Hearts were collected on D8 and D10 and analyzed for VEGF mRNA by quantitative RT-PCR. Hypoxia induced a 169 ± 35% and 200 ± 31% increase in cardiac VEGF mRNA on D8 and D10, respectively. We then determined the effects of TCDD on cardiac VEGF expression at these same time points. Fertile chicken eggs were injected with control or 0.24 pmol TCDD/g on day 0; hearts were collected on D8 and D10, and analyzed for VEGF mRNA by quantitative RT-PCR. TCDD significantly reduced VEGF mRNA on D8 by 25 ± 1.5%, but had no effect on D10. These results demonstrate that cardiac VEGF expression during coronary angiogenesis is regulated, in part, by hypoxia and further suggest that TCDD can reduce VEGF mRNA during coronary development. Future studies will evaluate the ability of TCDD to inhibit hypoxia-induced VEGF mRNA on D8 and D10. Supported by NIH ES09804.

548 TOXICITY OF CB 126 IN METAMORPHOSING SUMMER FLOUNDER LARVAE.

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The summer flounder, *Paralichthys dentatus*, is a commercially important species of flatfish found on the East Coast of the United States, from Cape Hatteras to Maine. Pelagic, stomachless larvae metamorphose into the benthic, gastric, juvenile flatfish in shallow coastal bays, lagoons and estuaries during the spring. In summer flounder, metamorphosis is regulated by thyroid hormones (TH), and chemically induced hypothyroidism inhibits metamorphosis past the very early stages. PCBs are among the major pollutants found in industrially impacted coastal ecosystems, and are known to act as endocrine disrupters by lowering blood TH levels in mammals and some fish. This project is concerned with determining the developmental effects of the dioxin-like CB 126 on metamorphosing larvae, and ultimately to understand if any of the effects are mediated by thyroidal disruption. Here we present results of the first experiments carried out 30 day-old (premetamorphic) and 39 day-old, (early metamorphosing) larvae to establish their sensitivity to CB 126. Larvae were exposed to radiolabeled CB 126 through the water, and grown out for 3 weeks to assess mortality. Cytochrome P450 IA1 immunoreactivity was measured as a marker of Aryl hydrocarbon Receptor pathway activation in liver, small intestine, stomach, and kidney. Mean metamorphic stage was calculated at the end of growout to determine if sublethal doses had an effect on metamorphosis. The younger group appeared more sensitive than the older (LD 20 = 20.9 pg/mg and 170 pg/mg, respectively). Sensitivity to CB 126 was similar to that observed in embryos of other marine fish (e.g. *Fundulus heteroclitus*). CYP1A1 induction was highest in the kidney and liver, lowest in anterior intestine, and was maximal near the LD 20 in all tissues; this pattern was in accord with observations in other species. In the younger group we observed a dose-dependent trend toward more advanced metamorphic stage, suggestive of an effect of CB 126 on developmental rate.

549 EXPRESSION OF A TRUNCATED RETINOIC ACID RECEPTOR ALPHA IN PHENYTOIN-INDUCED CLEFT PALATE.

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The anticonvulsant drug phenytoin (PHT) has been widely studied with respect to its teratogenic potential to induce orofacial clefts in the offspring of epileptic patients. The hypothesis that PHT-induced malformations involve perturbations in retinoid signalling pathways subsequent to altered gene expression, and, presumably, altered protein expression, was investigated in the current studies. Using *in situ* transcription/antisense RNA amplification techniques, and reverse Northern blots, our laboratory has previously shown that *in utero* exposure to PHT alters expression of the retinoic acid receptors (RARα, RARβ, and RARγ) in the developing embryonic palate. In the current study, alterations in expression of the RARα protein were examined immunohistochemically in the craniofacies of murine embryos following chronic *in utero* exposure to PHT. Using a commercial antibody to the C-terminal end of the RARα protein, and a custom antibody to the N-terminal end of the protein, we were able to demonstrate expression of a C-terminal truncated RARα' protein in the craniofacial region of GD 14.5 PHT-exposed embryos presenting with a cleft palate phenotype. In control embryos, cells expressing the full-length RARα protein in the region of palatal fusion were undergoing an epithelial-mesenchymal transformation. However, in PHT-exposed embryos, epithelial cells on the medial palatal shelves expressed the truncated RARα' protein and did not undergo fusion, but rather, differentiated into columnar epithelial cells. RARα transcripts expressing alternate splice-site variants were quantified in a ribonuclease protection assay, and there was a significant increase in the alternately spliced mRNA coding for the truncated RARα' protein in the palates of PHT-exposed embryos. The relative increase in expression of a dominant-negative RARα' protein following PHT exposure may affect transcriptional regulation of downstream growth factors necessary for normal craniofacial/palatal morphogenesis.

550 ROLE OF FOLATE IN FUMONISIN-INDUCED NEURAL TUBE DEFECTS.

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The current studies investigate the mycotoxin fumonisinB1 (FB1) and its interaction with folate transport systems in the induction of neural tube defects (NTDs). FB1 is a contaminant of corn associated with a variety of diseases, and recent reports suggest it may act as a developmental toxicant. Experimental evidence indicates that exposure to FB1 adversely affects folate uptake *in vitro*, potentially compromising cellular processes dependent on this vitamin. Since maternal folate deficiency has been causally linked to NTDs, we investigated this interaction as a potential mechanism for FB1-induced NTDs in a murine model. Preliminary results using inbred strains of mice indicate a differential genetic susceptibility to FB1-induced NTDs. SWV mice were completely resistant to the teratogenic effects of FB1, whereas there was a dose-dependent increase in the incidence of NTDs in the susceptible LMBc strain. Pregnant LMBc dams injected with 20mg/kg FB1 ip on GD 7.5 and GD 8.5 had a significant number of pups present with NTDs (81%). The precise timing of exposure during gestation was also critical, since a single FB1 dose on GD 8.5 resulted in only 16% exencephaly. Folinic acid (50mg/kg) administered periconceptionally to pregnant dams by daily oral gavage was unable to provide protection and rescue the phenotype. FB1 inhibits the enzyme ceramide synthase, resulting in a decrease in biosynthesis of sphingolipids, important membrane components for GPI-anchoring of the folate receptor. Since depletion of cellular sphingolipids may disrupt folate receptor function, we examined FB1-induced alterations in folate transport by injecting pregnant dams with ^{3}H-folate and measuring folate uptake in embryos. Our results indicated that ^{3}H-folate uptake was significantly inhibited in FB1 exposed embryos compared to controls. Using ^{14}C-labeled FB1, we were also able to demonstrate that FB1 crossed the placenta, inhibiting folate uptake within the developing embryo, and suggesting folate deficiency as a mechanism for FB1-induced developmental toxicity.

551 INTERACTION BETWEEN PHENYTOIN AND RETINOIC ACID RECEPTORS.

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Phenytoin (PHT) has been one of the most widely studied of all therapeutic agents with respect to its potential to induce congenital malformations. In general, adverse pregnancy outcomes have been observed in approximately 8 to 10% of the infants exposed *in utero* to PHT. A syndrome consisting of a well-described pattern of both major and minor congenital defects have been ascribed to *in utero* PHT exposure and have been termed the Fetal Hydantoin Syndrome (FHS). One of the well-recognized characteristics of FHS is craniofacial dysmorphia. Although well studied, the precise mechanism(s) by which PHT alters normal craniofacial development remains unknown. It has been shown that PHT alters the expression of retinoic acid receptors in the developing craniofacial tissue. In the current study we investigated whether PHT might act as a retinoic acid receptor (RARα) agonist. Using lung tissue, a rich source of RARα, from a murine strain that is susceptible to the teratogenic effects of PHT, competitive binding studies were performed using [3H]- *all trans*-retinoic acid (*at*RA). PHT competed with *at*RA in a dose dependent manner. However, PHT was more effective in the presence of liver microsomal enzymes. Additionally, if epoxide hydrolase inhibitors were added to the microsomal enzyme mixture RA binding was greatly diminished. Since this suggests that a metabolite of PHT could bind to RARα, we wanted to determine whether PHT could affect genes that are regulated by *at*RA. Using a reporter cell line transfected with a RA response element (RARE), which was linked to a *lac-Z* gene, we show that PHT could induce the *lac-Z* gene. However, if these cells were cultured in the presence of liver microsomal enzymes, the induction of the *lac-Z* gene was significantly increased in a dose dependent manner from 50–3000uM of PHT. Collectively these data suggest that a metabolite of PHT, most likely an epoxide, could act as a RA-agonist and alter the gene expression, which could lead to the observed craniofacial malformations.

552 ANTIBODY PRODUCTION INDUCED BY FOOD PROTEINS IN MICE : DOSE RESPONSES.

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A limited range of produce, including peanuts, tree nuts and eggs, is responsible for the majority of IgE-mediated food allergies. We have examined the ability of food proteins of varying allergenic potential to provoke IgG and IgE antibody responses following systemic (intraperitoneal; ip) exposure to BALB/c strain mice. Animals were treated with a purified peanut allergen (Arachis hypogea lectin) or with ovalbumin (OVA), a major allergenic constituent of chicken egg. Mice were also exposed to proteins derived from the potato, a foodstuff for which there is little evidence of allergenicity; to the purified lectin (Solanum tuberosum), or to a crude potato protein extract (PPE). Animals (n=5 per group) received various concentrations of protein by ip injection on days 0 and 7. Seven days later, serum samples were analyzed for specific IgG content by enzyme-linked immunosorbant assay and for specific IgE content by homologous passive cutaneous anaphylaxis assay. Administration of OVA or peanut lectin provoked vigorous IgG antibody responses and relatively high titer IgE at all doses tested. Thus, titers ranged from 1/128 to 1/16 for 0.2% to 0.02% peanut lectin and from 1/62 to 1/16 for 10% to 1% OVA. Potato lectin (10% to 1%) stimulated similar titers of IgG antibody to those induced by administration of the same concentrations of OVA, but only induced low titer (positive with neat serum only) IgE at the maximum dose tested (10%). PPE was somewhat less immunogenic over the same dose range and also failed to stimulate marked IgE responses (titer of 1/2 at 10% only). Particularly taking into account the dose responses, these food proteins display a differential capacity to provoke IgE antibody which is consistent with their differing allergenic potential in man.

553 THE SENSITIZING POTENTIAL OF DIFFERENT FOOD PROTEINS IN AN ORAL BROWN NORWAY (BN) RAT FOOD ALLERGY MODEL.

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Brown Norway rats were sensitized with common strong and non-allergenic proteins to evaluate the potential predictive value for allergenicity of food proteins. Natural, purified peanut Ara h1, shrimp tropomyosin, hen egg-white ovalbumin, potato patatin (the major allergenic protein in an uncommon allergen), and beef tropomyosin (a non-allergenic protein), were tested. BN rats (n=6) were either gavaged daily with 0.01, 0.1, or 1 mg protein/ml/rat during 42 days, with or without the oral adjuvant Cholera Toxin (CT), or sensitized by intraperitoneal injection (ip) with alum as a positive control. Weekly serum samples were tested for antigen specific IgE and IgG2a (a Thelper-2 mediated subclass in rats) antibody levels using a passive cutaneous anaphylaxis assay and enzyme-linked immunosorbent assay (ELISA). Although, responses to oral sensitization were not as common as to ip injection with alum, an apparent gradation of responses was observed in animals dosed with 1 mg per day without CT. Fifty percent of the animals developed specific IgE responses to the strong allergens Ara h1 and Shrimp tropomyosin, 70% to ovalbumin, and one of six animals developed a specific IgE response to patatin. No animals produced IgE responses to beef tropomyosin. Interestingly Ara h1, shrimp tropomyosin and ovalbumin are all stable in pepsin at low pH, a trait that is common for food allergens, while patatin is quite labile. Addition of CT as an oral adjuvant at day 0 and 7 during sensitization did not consistently increase the number of IgE responding animals. The reported results suggest that in the BN rat the oral route of sensitization, without the use of CT as an oral adjuvant, may be more predictive for food allergens than injection of the antigen with alum. In addition, based on the obtained results measurement of protein-specific IgG2a is not likely to be used as a stand-alone parameter for allergic sensitization.

554 PEANUT PROTEIN ELICITS T HELPER 2 CYTOKINE PRODUCTION AS DETECTED BY GENE EXPRESSION AND PROTEIN SECRETION IN MICE.

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Sensitization to peanuts is associated usually with high titer IgE antibody, consistent with the selective activation of T helper (Th) 2 type cells. We have characterized cytokine profiles, at the mRNA and protein levels, induced by exposure of mice to a purified peanut allergen, *Arachis hypogea* lectin. Fourteen days following intradermal exposure of BALB/c mice to peanut lectin, draining lymph node cells (LNC) were isolated and cultured in the presence or absence of peanut lectin, concanavalin A (con A) or irrelevant protein for 24 to 120 hours. Control animals were untreated (naïve). Cell proliferation was assessed over the final 24 hours of the culture period. Culture supernatants were analyzed for cytokine protein secretion by Enzyme-Linked Immunosorbent Assay (ELISA) and cytokine gene expression was evaluated by ribonuclease protection assay (RPA) using probes for various Th1- and Th2-type cytokines. Treatment of control (naïve) LNC with peanut lectin (0.2 mg/ml) failed to stimulate marked proliferation, confirming that peanut lectin is non-mitogenic. Culture with con A (2 μg/ml) induced vigorous proliferation and transient expression of mRNA for the type 1 cytokines interferon γ (IFN-γ) and interleukin (IL) 2. Restimulation of LNC from peanut-primed mice with peanut

lectin and con A in each case induced vigorous proliferative responses. Culture with peanut lectin also induced the expression of high levels of transcripts for the Th2 cytokines IL-4, IL-5, IL-10 and IL-13 throughout the time course investigated. Conversely, a more Th1-type cytokine profile was observed following restimulation with con A, with high expression of IL-2 and IFN-γ after 24 hours of culture, and less vigorous production of type 2 cytokines. These expression patterns were paralleled by cytokine protein secretion profiles. These data confirm that exposure of mice to peanut allergen results in the selective stimulation of a Th2-type response.

555 DIFFERENTIAL ANTIBODY RESPONSES PROVOKED BY SYSTEMIC EXPOSURE OF MICE TO Ara h 1 AND Sol t 1.

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Most cases of IgE-mediated food allergy are associated with a relatively limited range of produce, including eggs, peanuts and shellfish. We have isolated and purified native patatin (Sol t 1), which represents approximately 40% of the total protein derived from the potato, a foodstuff which is recognized as a relatively rare cause of allergenicity, and vicilin from the peanut (Ara h 1). Antibody responses induced by systemic (intraperitoneal; ip) exposure of BALB/c strain mice to these proteins have been characterized. In four independent experiments, animals (n=5) received an ip injection of 0.25ml of a 1% solution of protein in phosphate buffered saline on days 0 and 7, with serum samples prepared for analysis 7 days later. Specific IgE antibody was measured by homologous passive cutaneous anaphylaxis assay and specific IgG antibody production was analyzed by enyzme-linked immunosorbant assays. In each experiment, exposure to Ara h 1 stimulated detectable IgE in the majority of animals (60% to 100%), with titers for pooled serum samples ranging from 1 in 4 to 1 in 16. Identical treatment with Sol t 1 resulted in only a minority of IgE responder mice in each experiment (0% to 40%) and pooled serum samples were uniformly IgE-negative (no detectable IgE with neat serum). In contrast, analyses of specific IgG antibody responses revealed that both Sol t 1 and Ara h 1 provoked vigorous IgG antibody production in all exposed mice with comparable titers achieved. Thus, under conditions where both proteins are of equivalent immunogenicity (with respect to IgG antibody responses), a divergent capacity to stimulate IgE antibody production is observed. The differential abilities of Ara h 1 and Sol t 1 to stimulate specific IgE antibody following systemic exposure to BALB/c strain mice are consistent with their differing potential to cause allergenicity in man.

556 CHANGES IN THE COMPLEMENT SYSTEM IN TRIMELLITIC ANHYDRIDE-INDUCED OCCUPATIONAL ASTHMA IN THE MOUSE.

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Trimellitic anhydride (TMA) is a small molecular weight chemical known to cause occupational asthma. Recent studies in animal models suggest that the complement system plays a role in mediating symptoms of asthma. To determine if complement changes accompanying eosinophilia into the lung differ in TMA-induced asthma compared to ovalbumin (OA)-induced asthma, parallel murine asthma models were used. BALB/c mice were sensitized as follows: days 1 and 3 intradermally with either TMA or OA in corn oil vehicle; day 12 intratracheally with either TMA conjugated to mouse serum albumin (TMA-MSA), OA, or MSA as control. To elicit the allergic response, mice were challenged intratracheally on days 19, 22, and 23 with either 30 μg or 400 μg TMA-MSA, OA or control MSA. Eosinophil infiltration and concentrations of complement component 3 (C3) or the complement activation product C5a in bronchoalveolar lavage (BAL) were assessed 72 hr later in 4 to 15 animals per group. Mouse C3 was determined by ELISA and mouse C5a by a Western blot method. Challenge of a sensitized animal with 30 or 400 μg of the corresponding antigen resulted in significant eosinophil infiltration into the lung compared to MSA challenge. After challenge with 30 μg antigen, the concentration of C3 in the BAL increased in OA challenged mice, but not in TMA-MSA challenged mice. However, with 400 μg antigen challenge, the C3 in the BAL increased after either OA or TMA-MSA challenge when compared to MSA challenged controls. A significant increase in C5a in the BAL was only apparent after challenge with 400 μg TMA-MSA compared to MSA challenged animals, with no changes observed in OA challenged mice. These data demonstrate that different antigens elicit different changes in the complement system, and suggest that the mechanism of the asthmatic response may differ with the antigen. (Supported in part by NIH ES 07406).

557 INTERLEUKIN-4 AND INTERLEUKIN-6 WERE INCREASED IN RATS SENSITIZED WITH TOLUENE DIISOCYANATE.

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In order to investigate the cytokine profile in toluene diisocyanate (TDI)-induced occupational asthma, we conducted a quantification of cytokine production in a murine model of respiratory hyperreactivity to TDI. Wistar rats were sensitized with intranasal application of 10% TDI and provoked with 5% TDI to induce airway hypersensitivity. The blood leucocytes were counted, and bronchoalveolar lavage (BAL) was performed and the cellular responses in BAL fluid were analysed. Lung histological examination was performed to investigate the inflammatory status in the airway. The production of IL-2, IL-4, IL-6 and IFN- in serum, BAL fluid and spleen cell were determined with ELISA kits. The cellular results demonstrated that neutrophils and eosinophils in blood were significantly increased and the total cells and each different cell, in particular eosinophils in BAL fluid were markedly increased in TDI sensitized rats. Histological analysis showed that a respiratory inflammation represented by prominent infiltration of eosinophils in central and peripheral airways was present in TDI-sensitized rats. The cytokine assays revealed that in TDI-sensitized rats, IL-4 was predominately secreted in serum, and IL-4 and IL-6 rather than IL-2 and IFN- were predominately secreted in BAL fluid and in spleen cell. These findings suggested that IL-4 and IL-6 are preferentially produced and may have an important role in occupational asthma induced by TDI.

558 SPECIES COMPARISONS AND STATISTICAL ANALYSES OF CYTOKINE FINGERPRINTING FOR CHEMICAL ALLERGENS.

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Prolonged topical exposure of BALB/c strain mice to different classes of chemical allergen induces cytokine secretion profiles consistent with the selective development of discrete functional subpopulations of T cells. Thus, treatment with the reference contact allergen 2, 4-dinitrochlorobenzene (DNCB), which apparently lacks respiratory sensitizing potential, elicits a preferential type 1 cytokine production pattern, whereas the reference respiratory sensitizer trimellitic anhydride (TMA) stimulates a selective type 2 phenotype. We have now compared allergen-induced cytokine secretion profiles in the Brown Norway (BN) rat with those stimulated in the BALB/c strain mouse. Under conditions where TMA and DNCB induced similar levels of immune activation, divergent cytokine expression patterns are elicited. TMA-activated LNC isolated from both species elaborated high levels of the type 2 cytokines interleukin (IL)-10 and IL-13, but little of the type 1 cytokines IL-12 or interferon γ. For vigorous IL-4 production, LNC from both BALB/c mice and BN rats required restimulation *in vitro* with the T cell mitogen concanavalin A. DNCB-stimulated LNC generally displayed the converse type 1 phenotype. Cytokine secretion profiles displayed by LNC derived from BN rats were considerably more variable than those observed for LNC from BALB/c strain mice. With the exception of IL-4, statistically significant differences ($p<0.01$) between TMA- and DNCB-activated LNC were recorded for all cytokines in BALB/c mice. For the BN rat, differences reached statistical significance ($p<0.01$) only for the expression of IL-13. These data demonstrate that the intrinsic ability of DNCB and TMA to promote preferential type 1 and type 2 responses, respectively, is species-independent. However, with respect to the identification of potential chemical respiratory allergens as a function of induced cytokine secretion profiles (cytokine fingerprinting) these data suggest that the use of the BALB/c strain mouse will provide the more robust method for hazard assessment.

559 PARA-PHENYLENEDIAMINE (PPD) REDUCES MONOCYTE CHEMOTACTIC PROTEIN-1 (MCP-1) LEVELS IN PPD/BB SPECIFIC T CELL CLONES, PBMC, MONOCYTES, AND U937 CELLS.

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Para-Phenylenediamine (PPD) is very strong allergen causing allergic contact dermatitis. We recently found that CD4+ T cells are involved in PPD contact allergy. PPD/BB specific T cell clones secreted high amounts of IL-4 and IL-5. However, little is known about the impact of chemokines in PPD allergy. Chemokines may play an important role in the development and exerbation of delayed hypersensitivity reactions (Rollins, 1997). One CC chemokine, the monocyte chemotactic pro-

tein 1 (MCP-1), has been implicated in chronic inflammatory diseases such as rheumatoid arthritis as well as allergic concact dermatitis. The possible role of MCP-1 in the PPD contact allergy has not been investigated so far. We analyzed the capacity of PPD and it's autooxidation product Bandrowski's Base (BB) to modify MCP-1 levels. PPD reduced MCP-1 secretion up to 80% compared to controls while BB did not. The latter was found using PPD/BB specific T-cell clones, PBMC's, monocytes as well as in a cell line (U937). Further analyis revealed that PPD reduced MCP-1 mRNA expression and mRNA stability. These results indicate that PPD can directly influence immune surveillance. This work was supported by the BMBF (IZKF BIOMAT, G11) and the European Community.

560 CYTOKINE PRODUCTION INDUCED BY LOW MOLECULAR WEIGHT CHEMICALS AS A FUNCTION OF THE STIMULATION INDEX IN THE LOCAL LYMPH NODE ASSAY: AN APPROACH TO DISCRIMINATE CONTACT SENSITIZERS FROM RESPIRATORY SENSITIZERS.

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In general, contact sensitizers have been shown to selectively induce Th1 immune responses, such as IFN-gamma production, whereas Th2 responses, such as IL-4 production, were seen after exposure to respiratory allergens. However, these features are to a large extent dependent on the dose of the particular allergen. Therefore, the aim of the present study was to investigate the distinction between sensitizers and respiratory allergens, by establishing dose-dependent cytokine profiles. The contact allergens 2, 4- dinitrochlorobenzene (DNCB) and hexyl cinnamic aldehyde (HCA), as well as the respiratory allergens phthalic anhydride (PA), toluene diisocyanate (TDI), and trimellitic anhydride (TMA) were tested. For a range of concentrations, both proliferative responses and cytokine production were established. Estimated concentrations were derived at several stimulation indices (SI's). From that, IFN-gamma, IL-4, and IL-10 production as a function of estimated concentrations were calculated. All three respiratory allergens showed significantly higher IL-4 and IL-10 production patterns compared to DNCB and HCA. A positive identification of DNCB and HCA as contact allergens on the basis of IFN-gamm production, was observed only at very high stimulation indices (SI>40) for DNCB and already at low SI's (SI>7) for HCA. This approach combines potency evaluation and discrimination between contact and respiratory allergens in a single short assay. We propose that by direct linkage of proliferation and cytokine production in a dose-response manner, distinguishing contact allergens from respiratory allergens may be improved compared to present approaches.

561 AN APPROACH FOR SAFETY ASSESSMENT TO MINIMIZE POTENTIAL FOR TYPE I ALLERGY FROM PROTEINS IN PERSONAL CARE (PC) PRODUCTS.

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This report summarizes an approach for supporting the safety assessment of proteins in PC products (cosmetics, skin, hair care products) to minimize potential for inducing Type I allergy. There is increasing use of proteins (e.g. soya, hydrolyzed wheat, plant extracts) in PC products. Currently there are over 3000 proteins, protein extracts listed in the INCI dictionary for commercial PC product use. Proteins can induce IgE mediated Type I hypersensitivity, that may lead to symptoms like urticaria and respiratory allergy. In general, use of proteins has been shown to be safe, and levels of proteins in formulations are usually low (<1% w/w). However, exposure to some proteins has caused allergy: urticaria *via* hair products with quarternized hydrolyzed collagen (Niinimaki 1998. Allergy 53:1073-87); urticaria *via* PC products with hydrolyzed wheat (Varjonen 2000. Allergy 55:294-96). A strategy for safety risk assessments of proteins is proposed using an example of a body lotion containing a protease. Aerosol exposures were measured during showering after dermal application of this product, that showed exposures of 8-111ng/m3 of the protease. Dermal deposition studies also showed that 50% of applied protein remained intact on skin for over 24 hours. A previous report showed levels of 3-29ng/m3 of a similar protein inducted 4 of 61 panelists after 6 months of daily shower exposure (Kelling et al., 1998 J. Allergy Clin. Immunol. 101:179-87). This proposed risk assessment approach depends on; (1) innate allergenic potency of the protein; (2) structural similarity to other proteins for SAR comparison (3) exposure determination *via* relevant biologic route; (4) product application and usage habits; (5) comparisons to benchmark. For the body lotion example used here it was concluded that this product was considered unsafe for consumers.

562 EFFECTS OF MANCOZEB ON IMMUNE SYSTEM IN AGRICULTURAL WORKERS.

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Ethylenebisdithiocarbamate pesticides (EBDTCs) may have immunostimulating effects in man.The aim of this study was to investigate the immune status of agricultural workers exposed to an EBDTC fungicide (mancozeb).Twenty-six healthy subjects entered the study, 13 agricultural workers and 13 unexposed control subjects.The exposure to mancozeb was assessed through the determination of the levels of urine excretion of the main EDTCs metabolite, ethylentiourea (ETU).The study of the immune function was performed on serum and blood samples collected before and after the work shift in the workers and only once (morning samples) in controls.The following parameters were measured:complete and differential blood count, serum immunoglobulins, complement fractions, autoantibodies, lymphocyte subpopulations.As for the functional assay, we measured the proliferative response to several lymphocytes mitogens (PHA, PWM, anti-CD3 and PMA plus ionomycin) and cytokine production after stimuli (TNF, IL-4, g-INF) in a whole blood assay. We found that agricultural workers resulted significantly exposed to mancozeb (median ETU in urine, exposed:= 2.5 mg/g creatinine;range=0, 04-95.2 mg/g.Controls: median 0.25 mg/g creatinine; range: 0.02-0.25 mg/g). As for the immune testing, the post shift assay showed an increase in the proliferative responses to PHA 0, 3 % (median=85, 490, range=13, 989-137, 742 cpm vs 35, 796, range=313-117, 230) in the exposed subjects, as well as to anti-CD3 (25 ng/mL) and PWM 2%. Relatively to cytokine production, we found an increase in g-INF, while IL4 and TNF decreased, suggesting a preferential Th1 cells activation. In summary, our results confirm that exposure to mancozeb has slight immunostimulatory effects and point out the possibility to monitor and reveal immunomodifications in workers occupationally exposed to potential immunotoxic compounds using a whole blood assay.

563 ANTISENSE INHIBITION OF TRANSLATION ELONGATION FACTOR-1 DELTA REVERSES THE ONCOGENIC POTENTIAL OF CADMIUM TRANSFORMED CELLS.

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The molecular mechanisms of carcinogenesis induced by the human carcinogen – cadmium, were investigated. Differential display analysis of gene expression revealed the overexpression of translation elongation factor-1delta sub-unit in BALB/c-3T3 cells transformed with cadmium chloride compared with the non-transformed control cells. cDNA cloning (GenBank Accession Number AF304351), expression of the encoded protein, and functional analysis of cDNA transfected NIH3T3 cells indicated that translation elongation factor-1 delta is a proto-oncogene. In order to confirm the oncogenic potential of translation elongation factor-1 delta, BALB/c-3T3 cells transformed with cadmium chloride were transfected with the plasmid DNA expressing antisense TEF-1 delta mRNA. Expression of the antisense translation elongation factor-1 delta mRNA resulted in marked reversal of the oncogenic potential of the cadmium-transformed BALB/c-3T3 cells. This was evidenced by a significant reduction in the number of anchorage independent colonies developing from the transformed cells expressing antisense mRNA for translation elongation factor-1 delta compared with the vector alone-transfected control cells. Furthermore, sub-cutaneous injection of the antisense mRNA expressing transformed BALB/c-3T3 cells resulted in a significant reduction in the onset as well as the size of tumors in athymic nude mice. These results demonstrate that the cell transformation and tumorigenesis induced by the human carcinogen – cadmium, were due to the overexpression of translation elongation factor-1 delta - a novel cadmium-responsive proto-oncogene.

564 APPLICATION OF GENE ARRAY TECHNOLOGY TO TRANSCRIPTIONAL FINGERPRINTS OF PRIMARY RAT HEPATOCYTES EXPOSED TO CADMIUM ACETATE.

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Traditional toxicological testing utilizes whole animals to test potential toxicity of chemicals. These tests are expensive, time consuming, and provide little data on early low dose effects and gene expression in target cells. Gene chip technology allows the simultaneous monitoring of expression levels of thousands of genes and

can be used as a rapid and highly sensitive detector for toxicity evaluation and prediction. Cadmium (Cd) is an extremely toxic trace metal of concern to the US Air Force. Although toxic effects of Cd have been well characterized, its temporal effect on the transcriptome has not been studied. To determine the transcriptomic fingerprints of Cd toxicity, primary rat hepatocytes were exposed to Cd at 0, 1.25 and 2 μM for 2 hrs at 37 °C. Total RNA was isolated at -2 (pretreatment control), 0, 3, 6, 12 and 24 hrs post-exposure to determine mRNA expression profiles using the Affymetrix RatTox U34 array. Simultaneously, hepatocyte viability was assessed by LDH leakage and MTT reduction. Our results indicated that (1) Cd resulted in a significant time-course, dose-dependent induction of stress genes (ie., *hsp*70s, heme oxygenase, GLCLc, MT1 and NQO1); (2) the *hsp*70s displayed a differential dose-dependent up-regulation due to Cd treatment: at 3 h-76 fold/5 fold; at 6 h- 67 fold/8 fold (data are expressed relative to controls for the 2μM/1.25μM treatments); (3) 2 μM Cd down-regulated CYP450s, vimentin, and tyrosine aminotransferase at certain time points; (4) effects of Cd on gene expression profiles in rat primary hepatocytes were consistent with the results of cytotoxicity data, however, transcript responses were more sensitive. In summary, Cd-exposed primary rat hepatocytes give rise to a unique gene expression fingerprint. Modulation of gene expression will help elucidate the mechanisms of Cd toxicity and identify early biomarkers that can be used to predict human health.

565 EXPRESSION OF A HEME OXYGENASE-LUCIFERASE (HO-LUC) CONSTRUCT IN TRANSGENIC MICE FOLLOWING TREATMENT WITH DIFFERENT CHEMICALS.

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Heme oxygenase-1 (HO) is a key enzyme in the conversion of heme to bilirubin and may be involved in some cellular anti-oxidative responses. The HO gene has been previously shown to be rapidly up-regulated after treatment with chemicals or other inducers of oxidative stress. FVB mice transgenic for the luciferase gene under transcriptional control of the HO promoter (HO-luc) have been shown to express the HO-luc transgene similarly to the endogenous HO gene following the administration of HO-inducing compounds. *in vivo* tissue-specific expression of HO-luc can be visualized using a low-light imaging system (IVIS™) after i.p. injection of luciferin. The HO-luc mouse has previously been shown to express luciferase in response cadmium chloride, a potent HO inducer. In this study, compounds representing different toxicity pathways were used to evaluate the HO-luc Tg mouse as an *in vivo* screening tool for toxicology. Adult male and female HO-luc mice were treated (3-28 days) with acetaminophen (250 mg/kg, oral or ip), cadmium chloride (CdCl2, 10 μM/kg, ip), chloroform (100, 200 and 250 mg/kg oral or ip), clofibrate (500 mg/kg oral or ip), or doxorubicin (10 mg/kg intravenous). All compounds induced HO-luc expression with the greatest responses after CdCl2 or chloroform treatment, and tissue-specific pathology was confirmed. These findings suggest that the HO-luc mouse may be used as a model for *in vivo* screening of new and unknown compounds for their potential toxic effects.

566 UTILITY OF HISTORICAL DYNAMIC RANGE DATA IN MICROARRAY GENE EXPRESSION ANALYSIS.

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A major difficulty in applying microarray gene expression (MGE) data to toxicity assessment is interpretation of the significance of a gene's induction or repression. One important component to the dimension of significance is the response of genes in relation to their individual dynamic ranges of expression, which can differ significantly due to both biological factors and technical limitations within microarray platforms. In this study, we assess the utility of normalizing fold induction (FI) data based on individual gene dynamic ranges in the analysis and interpretation of MGE experiments with $CdCl_2$ and other compounds reputed to exert pro-oxidant (OX) effects (clofibrate, cyclosporin A, and puromycin aminonucleoside). Historical maximum (max) or minimum (min) FI values were derived from the Phase-1 database of *in vivo* rat MGE experiments, which includes 38 well-characterized, paradigm toxicants. FI data for each gene were then converted to % max or % min values by referencing corresponding historical values. In 6 hour liver samples from experiments with 3 rats treated with a single IP injection of 1 mg/kg $CdCl_2$, the most highly induced (> 2.5 FI in at least 2 rats) genes implicated in oxidant defense (OD) were heme oxygenase-1 (HO-1) (3.3, 3.1, 4.9) and metallothionein-1 (MT-1) (9.0, 21.5, 12.5). However, when % max values were considered, the most highly induced (> 40% of max in at least 2 rats) OD genes were Cu/Zn superoxide dismutase (Cu/Zn-SOD) (13%, 88%, 74%), heme-binding protein 23 (HBP23) (23%, 63%, 83%), and thiol-specific antioxidant (TSA) (6%, 45%, 82%). Induction values for both HO-1 and MT-1 were < 25% max for all 3 rats. Additionally, historical max data showed several instances of co-induction of Cu/Zn-SOD, HBP23, and TSA in liver by the other OX compounds. The results suggest that converting MGE data to values based on historical dynamic ranges can enhance the identification of genes that show substantial participation in specific toxicological responses, as well as the compounds that elicit those responses.

567 MAP KINASE PATHWAY DECREASES THE INDUCTION OF METAL RESPONSE ELEMENT-DRIVEN LUCIFERASE ACTIVITY BY CADMIUM.

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Both cadmium (Cd++) and zinc (Zn++) activate metallothionein (Mt1) gene transcription *via* the metal response element (MRE motif), to which the MRE transcription factor-1 (MTF1) binds. In cells cultured in low Zn++, most MTF1 is localized to the cytoplasm and is inactive. However, even under these circumstances, a modest percentage of MTF1 is in the nucleus and is binding-competent. Activation of Mt transcription by Zn++ is preceded by a stoichiometric activation of MTF1 binding and its nuclear accumulation. On the other hand, Cd++ activates MTF1-dependent Mt transcription without an increase in MRE binding or nuclear translocation. Thus, a modest amount of nuclear MTF1 may acquire potent transactivation potential in the presence of Cd++. Because heavy metals appear to activate oxidative stress-signaling, we assessed the role of signaling components of mitogen-activated protein (MAP) kinase pathways in controlling the trans-activity of MTF1. Transient transfection experiments were performed in monkey kidney fibroblast CV1 cells, using a reporter construct containing 153 bp of the Mt1 promoter fused to the luciferase (LUC) gene. Cd++ treatment resulted in a dose-dependent increase in LUC activity reaching a maximal 80-fold above control. Co-transfection with constitutively active MEKK1, an upstream regulator of the MAPK kinase pathway, shifted the dose-response to Cd++ approximately 4-fold to the right and decreased to half the maximal induction. MEK1 and MKK6 are the downstream mediators of MEKK1. Co-transfection of their constitutively active forms similarly decreased the cadmium-mediated induction as MEKK1. PD98059, a generalized inhibitor of MEK, augmented Cd++ induction, either with or without MEKK1 plasmid co-transfection. Using transient transfection with MTF1-null cells, these effects were found to be MTF1-dependent. [a]Supported, in part, by NIH grants P30 ES06096 and R01 ES10416.

568 DIFFERENTIAL EFFECTS OF ARSENIC, CHROMIUM, CADMIUM, NICKEL AND MITOMYCIN C ON GENE EXPRESSION AS MEASURED BY DNA MICROARRAY.

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Examining global effects of toxins on gene expression can be useful for examining patterns of biological response, underlying mechanisms of toxicity, and identification of candidate metal-specific genetic markers of exposure and response. Using gene microarrays, we examined changes in gene expression following low dose, acute exposures of Cd, Cr, Ni, As or mitomycin C (MMC) in BEAS-2B human bronchial epithelial cells. Treatments were chosen that did not cause any overt signs of toxicity, or changes in cell survival or replication as measured by long term colony forming assays. Total RNA was isolated from cells exposed to 3 μM Cd(II) (as cadmium chloride), 10 μM Cr(VI) (as sodium dichromate), 3 μg/cm2 Ni(II) (as nickel subsulfide), 5 μM or 50 μM As(III) (as sodium arsenite), or 1 μM MMC for 4 h. Gene expression profiles in response to these treatments were measured using a Clontech Atlas Human 1.2 cDNA microarray. A signal intensity threshold ratio of 1.5 was used for Cd, Cr, Ni and MMC, but a higher threshold value of 1.7 was used for As due to the much larger number of As-affected genes. Surprisingly, only a small subset of genes was differentially expressed in response to each agent: Cd, Cr, Ni, As (5 μM), As (50 μM), and MMC each differentially altered the expression of 25, 44, 31, 110, 65, and 16 individual genes, respectively. Interestingly, only a few genes were commonly expressed among the various treatments. Only one gene was altered in response to all four metals (hsp90), and no gene overlapped among all five treatments. Only three to seven genes overlapped among any two treatments, and similarly only a few genes were common to any three treatments. We also compared low (5 μM, non-cytotoxic) and high dose (50 μM, completely cytotoxic) arsenic treatments, which surprisingly affected expression of almost completely non-overlapping subsets of genes, suggesting a threshold switch from a survival response to an apoptotic response. (NIEHS (SBRP) ES07373)

569 MICROARRAY AND DIFFERENTIAL DISPLAY ANALYSES OF BONE CELL GENE EXPRESSION EARLY AFTER CADMIUM GAVAGE TO MICE.

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We developed an *in vivo* model for Cd-induced bone loss in which mice excrete bone mineral in feces starting 8 h after Cd gavage. The bone response starts at 5 μg Cd/l blood, below US OSHA guidelines for industry. Female mice of 3 strains

[CF1; metallothionein-wildtype (MT+); MT1, 2-deficient (MT-)] were placed on low Ca diet for 2 weeks. Each mouse was gavaged with 200 µg Cd or vehicle only. Fecal Ca was monitored daily for 8 days, starting 3 days before Cd gavage, to document bone response. For CF1 mice, bones were taken from 4 groups: +/- Cd, 2h after Cd; +/- Cd, 4h after Cd. MT+ and MT- strains had only +/-Cd, 4 h after Cd. PolyA RNA was isolated from marrow-free shafts of femura and tibiae, and each +/- Cd pair was submitted to Incyte Genomics for microarray analysis. Other RNA samples (4 h after Cd) were subjected to conventional DDRT-PCR. Fecal Ca results show that the 3 mouse strains differed in amount of bone Ca excreted after Cd (CF1 0.24±0.08 mg; MT+ 0.92±0.22 mg; MT- 1.7±0.4 mg). Differential display results show that 2 of 4 cloned bands that had confirmed Cd down-regulation by Northern analysis were mitochondrial. Another was 71% identical to arg-tyr kinase (GenBank U40827); the other was 74% identical to 2 mouse cDNAs of unknown function. Microarray results show that nearly all arrayed genes were unaffected by Cd. However, MT1 and MT2 had +Cd/-Cd expression ratios >1 in all four groups, while all ratios for MT3 were essentially '1', showing specificity. Both probes for MAPK 14 had expression ratios >1, while no other MAPK responded to Cd. Vacuolar proton pump ATPase and integrin αv (osteoclast genes), transferrin receptor, and src-like adaptor protein genes were stimulated by Cd; other src-related genes were unaffected. Genes down-regulated by Cd included procollagen type IX, and RIKEN cDNA 2900017D14. Results support the hypothesis that Cd stimulates bone demineralization *via* a MAPK pathway involving osteoclast activation.

570 APPLICATION OF CDNA MICROARRAY TO STUDY SUBACUTE CD TOXICITY.

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Following a single intraperitoneal injection of 2mg/kg CdCl2, there was a sequence of change in mouse liver which included a change in the level of MT-mRNA, MT protein, as well as a marked increase in the content of Zn, which was found to bind to the high molecular weight proteins fraction (Yang et al., 2000). It was suggested that the marked increase in tissue Zn might be related to events such as further subcellular damage or recovery. The aim of the present study is to identify possible role of Zn during the subacute phase of Cd induced injury by finding the genes that might be involved in the subsequent biochemical changes. Six animals were randomly paired. One of the animals in each pair was injected with 0.9% NaCl and the other with CdCl2 (2 mg/kg). The animals were sacrificed after 48 hr. The liver was removed and total RNA was extracted by TRIZOL reagent. Total RNA (100µg) from the control and treatment groups were labeled with Cy5 and Cy3 fluorescent dye, respectively. The labelled probes were hybridized with the UCLA mouse 2K cDNA chip which consists of 1920 spots at 65OC overnight. The arrays were washed with 2X SSC, 0.1% SD and 0.2X SSC buffer and scanned by the GSI Lumonics confocal laser scanner. Fluorescent signal intensities of each spot on the slide were further normalized by the total intensity normalization method. Results from different trials indicated that several genes include alpha-2-macroglobulin, ferrtin heavy chain, translocator of inner mitochondrial membrane 44, metallothionein 1, cytochrome P450 steroid inducible 3a1, N-myc downstream regulated 1 and some ESTs geneswere up-regualted. Amongst them, only alpha-2-macroglobulin were consistently induced by more than 2-fold in each case. We are in the process of confirming their statement levels by RT-PCR or Northern blot. Yang et al, (2000). Toxicology 154, 103-111.

571 METALLOTHIONEIN GENE AND PROTEIN EXPRESSION IN A HUMAN UROTHELIAL CELL LINE EXPOSED TO CADMIUM CHLORIDE.

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Metallothionein (MT) proteins are involved in the cellular stress response to toxic insult, and many bladder disorders have an environmental basis. The goal of this study was to characterize the expression of the MT isoforms in response to the classic MT inducer/toxic agent, cadmium, in UROtsa cells. UROtsa cells are immortalized human urothelial cells that exhibit many features consistent with normal human urothelium. One such feature is the exclusive basal expression of MT-1E, -1X and -2A genes. Acute exposure of UROtsa cells to 53.4 µM cadmium chloride for four hours resulted in the induction of MT-1E, -1X and -2A genes, as well as the MT-3 and -4 genes. Other metallothionein isoforms, MT-1A, -1B, -1F, -1G and -1H were not expressed at levels detectable by RT-PCR. Simultaneous detection of MT-1 and MT-2 proteins (MT-1/2) using the E-9 antibody showed an increase in the levels of MT protein corresponding to the increase in MT-1E, -1X, and -2A mRNA. Chronic exposure of UROtsa cells to 1, 5 and 9 µM cadmium chloride over a period of sixteen days, showed an increase in MT-1/2 protein, as well as an increase in MT-1E, -1X and -2A gene expression. MT-4 was induced in cells treated with 5 and 9 µM cadmium over the 16 day time course, whereas MT-3 mRNA was not detected in any of the chronically treated cells. These results suggest that MT- 1 and -2 are induced by acute and chronic exposure to cadmium, whereas MT-3 is only induced by acute exposure to cadmium.

572 MICROARRAY ANALYSIS OF MOLECULAR CHANGES SPECIFIC TO CADMIUM-TRANSFORMED HUMAN PROSTATE EPITHELIAL CELLS.

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Cadmium (Cd) is a carcinogenic metal implicated as a human prostatic carcinogen based on epidemiological studies, rodent studies and *in vitro* experiments. The molecular events underlying Cd carcinogenesis remain elusive, although our recent development of a Cd-transformed human prostate epithelial cell line (CTPE) provides a model amenable to molecular dissection. We initiated molecular comparisons of CTPE with the non-tumorigenic parental line (RWPE-1) as well as *ras*- (RWPE2-W99) and MNU-induced transformants derived from the same line. The goals of this study were to identify gene expression changes specific to CTPE cells in order to shed light on the carcinogenic mechanism of Cd and identify possible biomarkers of Cd-induced prostate tumors. This analysis revealed 29 genes whose expression is specifically altered in CTPE cells, including caveolin-1 and -2, P-cadherin, IL-6 and cytokeratins 8 and 18. Western blot analyses revealed that protein levels of caveolin-1and -2 and P-cadherin were elevated in CTPE cells, confirming the array results. Caveolin-1 was present in CTPE while undetectable in the other cells and caveolin-2 levels were increased 5-fold compared to RWPE-1 and 8-fold compared to RWPE2-W9. Caveolin-1 has been proposed as a marker of prostate tumor progression and the observed increase is consistent with the invasive and metastatic nature of CTPE-derived tumors. P-cadherin protein levels in CTPE cells were 3.4-fold those in RWPE-1 and 2-fold of those in RWPE2-W99. A review of the literature revealed no reports of increased P-cadherin in prostate cancer, making it a promising candidate for a specific biomarker of Cd-induced prostate tumors. We are expanding our study in order to better define the molecular changes specific to CTPE cells and beginning to construct a molecular fingerprint of Cd-induced prostate tumors. Development of such a fingerprint would dramatically impact the ability to assess the risk of Cd-induced prostate cancer in human populations.

573 ACUTE CADMIUM EXPOSURE INDUCES STRESS-RELATED GENE EXPRESSION IN WILD-TYPE AND METALLOTHIONEIN-I/II NULL MICE.

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This study examined effects of acute cadmium (Cd) on stress-related gene expression and free radical generation in wild-type and metallothionein-I/II-null (MT-null) mice. Atlas Toxicology arrays showed that Cd (40 µmol/kg as $CdCl_2$, ip for 3 hr) markedly increased the expression of genes encoding heat shock proteins, heme oxygenase-1, and DNA damage/repair response genes, such as DNA-damage inducible gene GADD45 and GADD153, DNA excision repair genes ERCC1, Rad23, Rad50, DNA mismatch repair gene PMS2, MSH3 and DNA glycosylases. The expression of genes encoding cytochrome P450 enzymes, UDP-glucuronosyltransferases, Mn-superoxide dismutase and catalase, was suppressed by Cd. MT-null mice were more sensitive than wild-type mice to Cd-induced stress-related gene expression, in accord with greater activation of transcription factor AP-1 and phosphorylated c-Jun NH_2-terminal kinase (JNK1/2) and extracellular signal-regulated kinase (ERK1/2). Thus, absence of MT exacerbates Cd-induced aberrant gene expression. To evaluate free radical production, mice were simultaneously given the spin-trap agent, N-*tert*-butyl-α-phenylnitrone (PBN, 250 mg in DMSO/kg, ip) with Cd, and livers were removed 30 minutes later for PBN-trapped radicals extraction with chloroform:methanol (2:1). The PBN-trapped radicals were detected with electron spin resonance. Cd treatment caused free radical generation in the liver of both wild-type and MT-null mice. Lipid peroxidation, as determined by hepatic concentrations of malondialdehyde and 4-hydroxyalkenals, was also increased in both wild-type and MT-null mice to a similar extent. These results indicate that a deficiency in MT enhances stress-related gene expression in response to Cd, but that the effect of MT may be down-stream of radical production.

574 SIGNAL TRANSDUCTION PATHWAYS IN CADMIUM-MEDIATED METALLOTHIONEIN-1 EXPRESSION.

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The purpose of this study was to investigate signal transduction pathways that participate in cadmium-induced regulation of metallothionein (MT), a thiol-rich metal binding protein with free radical scavenging properties. Using quantitative real-time RT-PCR, we show that *in vitro* cadmium (Cd) treatment of alveolar epithelial cells causes both time- and dose-dependent changes in steady state levels of MT-1. The expression of MT-1 was significantly elevated above control (25-fold) 4 hours after the addition of 10 μM $CdCl_2$. A maximum increase in MT gene expression (~ 100-fold) was achieved by hours. Transcript levels remained above control at 16 h (30-fold) and eventually returned to baseline levels by 24 hours. Exposure of cultures to 5, 10, or 20 μM $CdCl_2$ for 8 hours resulted in increases of MT expression of 25-, 100-, and 210-fold, respectively. Inhibitors, specific to protein kinase C (PKC), protein kinase A (PKA), ERK kinase (MEK), p38 mitogen activated protein kinase (MAPK), and tyrosine kinases, were subsequently tested to determine how each affected Cd-mediated induction of MT-1. In these experiments, alveolar epithelial cell cultures were pre-treated for 30 min with each inhibitor prior to an 8 hour treatment with 10 μM $CdCl_2$. Induction of MT by Cd was reduced by 70% when PKC activity was suppressed with the inhibitor GF190203X. In contrast, inhibition of MEK with PD90859 resulted in a 50% increase in Cd-mediated MT gene expression. Inhibitors of p38 MAPK and tyrosine kinase caused less dramatic changes in MT mRNA. In conclusion, the results of this study indicate that signal transduction pathways that include PKC and MEK as active components are of importance in the induction of MT-1 by Cd.

575 ANTISENSE-BCL-2-INDUCED SELECTIVE OXIDATION OF PHOSPHATIDYLSERINE IN NCI-H226 CARCINOMA CELLS: ROLE IN APOPTOTIC SIGNALING.

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The major function attributed to Bcl-2 is its ability to confer resistance against apoptosis. We have previously shown that Bcl-2 was effective in preventing of membrane phospholipid oxidation during apoptosis induced by a number of oxidants as well as non-oxidative stimuli. In particular, phosphatidylserine (PS) was protected against apoptosis-associated peroxidation and subsequent PS-dependent signaling events such as PS externalization. To further investigate the antioxidant/anti-apoptotic role played by Bcl-2 in oxidation of phosphatidylserine during apoptosis, we decreased the expression of Bcl-2 in the lung squamous carcinoma cell line NCI-H226 using a synthetic phosphorothioate oligonucleotide targeted against bcl-2 mRNA (antisense-bcl-2). NCI-H226 cells (106) were incubated with media only or with either the antisense-bcl-2 (0.3 M) oligonucleotide or with a specificity/cytotoxicity nonsense (0.3 M) oligonucleotide control for 5 h at 37C. Incubation of NCI-H226 cells in the presence of the antisense-bcl-2 oligonucleotide resulted in a significantly increased amount of apoptotic cells as compared to controls. Using our technique to assess oxidative stress using cis-parinaric acid (PnA), we found a significant oxidation of Pn-A-PS in apoptotic Bcl-2-depleted NCI-H226 carcinoma cells. No significant differences in oxidation of PnA-labeled phosphatidylcholine and phosphatidylethanolamine between control cells and cells after treatment with both nonsense and antisense-bcl-2 was found. This selective oxidation of PS cannot be attributed to changes of phospholipid composition as our HPTLC analysis of the major phospholipid classes showed no differences in the phospholipid composition between control (untreated) cells and cells incubated in the presence of either antisence-bcl-2 and nonsense oligonucleotides. We conclude that Bcl-2 plays an important role in regulation of programmed cell death and PS oxidation during apoptosis.

576 ROLE OF APOPTOSIS INDUCTION IN DEVELOPMENT OF LUNG INJURY FROM HIGH BURDENS OF TITANIUM DIOXIDE.

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We previously examined the role of apoptosis in lung pathology due to silica, a highly toxic particle and demonstrated that apoptotic cells and degradation products significantly accumulate as injury develops. In this study we test the hypothesis that titanium dioxide (TiO2), a particle with no known specific toxicity, induces apoptosis and accumulation of apoptotic degradation products. To test this, male, F344 rats were given intratracheal instillations (IT) of 0.4, 4 and 40 mg of TiO2. At 1 day, 1 week and 4 weeks after instillation rats were sacrificed, lungs preserved by intra-tracheal instillation of fixative. Sections were processed for TdT labeling of apoptotic nuclei with a fluorescent indicator and counter-stained to label non-apoptotic nuclei. The number of apoptotic cell nuclei per lung was measured by morphometric methods. Additional sections were stained with Sirius Red to detect areas of collagen accumulation. Apoptotic cells in the saline instilled lungs were minimal at 1 and 4 weeks (0.2±0.2 and 0.3±0.4 million cells per lung, mean±SE). One week after IT, apoptotic cells per lung were 1.9±0.1, 4.4±0.4 and 13.3±2 million in the 0.4 mg, 4 and 40 mg groups respectively. At 4 weeks the number of apoptotic cells was increased to 5.2±0.5, 9.5±2.0 and 24.1±3.1 million in the 0.4, 4 and 40 mg TiO2 groups. Sirius Red staining of lung sections demonstrated areas with significant connective tissue accumulations in response to the IT of TiO2 in both the 4 and 40 mg TiO2 groups at 4 weeks of exposure. Sirius Red staining of collagen in saline and 0.4 mg TiO2 groups was normal at 1 and 4 weeks. Our results demonstrate that high levels of inert, non-toxic dusts produce significant numbers of apoptotic cells and products. At high levels of lung burden the induction of apoptosis is associated with development of fibrotic foci. The results suggest that injury from the accumulated apoptotic products may be responsible for injury observed in high lung burden exposures to particulates such as TiO2 which have no specific toxicity.

577 BENZO(A)PYRENE 7, 8-DIHYDRODIOL IS GLUCURONIDATED BY HUMAN UGT1A9 AND CAUSES APOPTOSIS IN HEPG2 CELLS.

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The human UDP-glucuronosyltransferases (UGTs) are involved in cellular detoxification and have been implicated as a mechanism to promote genoprotection. Polycyclic aromatic hydrocarbons such as BENZO(A)PYRENE (BP) are metabolized through oxidation and epoxidation to simple and complex phenols. Both phenolic and dihydrodiol metabolites have been shown to be proximate mutagens capable of being activated to ultimate forms. While most of the simple phenols are substrates for glucuronidation, little is known about the more complex dihydrodiols. Using expressed human UGTs (1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4 and 2B7), we have demonstrated that BP-*trans*-7, 8-dihydrodiol (±) (7, 8-diol) is actively glucuronidated by UGT1A9. In cells deficient in UGT1A9, such as HepG2 cells, experiments were conducted to examine the mutagenic properties of 7, 8-diol on cell viability. Annexin V analysis revealed in a dose dependent fashion that HepG2 cells were undergoing apoptosis following treatment with various concentrations of the 7, 8-diol. DNA fragmentation assays confirmed the presence of internucleosomal cleavage products consistent with the analysis of poly (ADP-ribose) polymerase (PARP) as shown by the appearance of the PARP 89 kD fragment. In Western blot analysis, HepG2 cells treated with 2.5 μM 7, 8-diol led to a two fold increase in cytosolic Cytochrome C, consistent with one of the properties of apoptosis. $Bcl\text{-}_{XL}$, an anti-apoptotic protein, decreased over 50%, while Bax, a pro-apoptotic protein, increased to 150% of that following vehicle treatment. In addition, an extensive down-regulation of xIAP (inhibitors of apoptosis) was detected which paralleled the appearance of apoptotic DNA fragments. In conclusion, HepG2 cells may be a sensitive biological tool to examine the actions of mutagens on apoptosis in addition to monitoring the genoprotective role of the human UGTs on cell viability. (Supported by USPHS grant CA79834)

578 INVOLVEMENT OF OXIDATIVE STRESS IN SDZ IMM 125-INDUCED APOPTOSIS IN RAT HEPATOCYTES.

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The mechanisms underlying the apoptotic activity of the immunosuppressive drug cyclosporine A and its hepatotoxic O-hydroxyethyl-D(Ser)8-cyclosporine derivative SDZ IMM125 in rat hepatocytes are not fully understood. It was the purpose of the present study to investigate the role of oxidative stress in SDZ IMM125-induced apoptosis, and to evaluate the role of caspase-3 by coincubation of anti- and pro-oxidants with rat hepatocytes. SDZ IMM 125 induced a dose-dependent increase in chromatin condensation and fragmentation, and the activation of caspase-3. Supplementing the cell cultures with the antioxidants, DL--tocopherol-polyethylene-glycol-1000-succinate and ascorbic acid significantly inhibited the SDZ IMM125-mediated increase in chromatin condensation and fragmentation, and caspase-3 activity. The reducing agent, dithiothreitol, significantly inhibited the SDZ IMM125-mediated increase in chromatin condensation and caspase-3 activity. The glutathione synthetase inhibitor, buthionine sulfoximine, significantly enhanced SDZ IMM125-mediated caspase-3 activation and increased SDZ IMM 125-mediated chromatin condensation and fragmentation. The present data suggest that SDZ IMM125-induced apoptosis is mediated by the imbalance of anti- and pro-oxidant steady state in rat hepatocytes and that the intracellular redox-state can act as a modulator of apoptosis. The results further suggest that antioxidants and pro-oxidants might act on apoptosis by modifying caspase-3 activity.

579 REVERSAL OF ACETAMINOPHEN (AAP) AND THIOACETAMIDE (TAM)-INDUCED CASPASE-ACTIVATED DNASE (CAD) AND OXIDATIVE STRESS-MEDIATED APOPTOTIC AND NECROTIC LIVER CELL DEATHS BY *MOMORDICA CHARANTIA* BIOFLAVONOIDS.

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Acetaminophen and Thioacetamide are well known for their liver-specific apoptogenicity. Similarly, bitter gourd or *M. charantia* extract (MCE), has been vouched nearly a century, for its anti-diabetic, antioxidant and numerous other medicinal properties. However, the influence of MCE on apoptosis-inducing signals *in vivo* remains unknown. This study investigated effects of MCE pre-exposure on AAP- and TAM- induced hepatotoxicity, and events that orchestrate apoptosis(oxidative stress and CAD-dependent genomic DNA fragmentation). Additional focus was to determine the incidence of apoptotic and necrotic deaths and the degree of cytoprotection. Male ICR mice were given orally MCE for 7 and 14 days. On 7th or 14th day, AAP (400 or 500 mg/kg) or TAM (25, 50, 100 mg/kg) was administered ip. All animals were sacrificed 24 hrs after AAP or TAM injections. Blood samples were collected to analyze serum chemistry, and liver tissues for biochemistry and histopathology. Results show that liver injuries induced by both AAP and TAM were significantly counteracted by MCE pre-exposures. In addition, apoptosis controlling events, such as orderly DNA fragmentation and oxidative stress (MDA accumulation and glutathione depletion) culminated by AAP and TAM were also significantly inhibited. Histopathological evaluation of liver sections by brightfield microscopy (X1000) revealed, both AAP and TAM caused dramatic glycogen depletion coupled with massive apoptotic cell death. Interestingly, MCE+AAP exposed tissues discerned near complete reversal of AAP-induced effects, whereas, MCE+TAM-treated sections exhibited significant protection from TAM-induced hepatotoxicosis. These findings suggest that, besides chemopreventive properties, MCE may influence drug and chemically-induced apoptogenic and necrogenic signaling mechanisms in the liver.

580 MOLECULAR MECHANISMS OF ZINC INHIBITION OF ETHANOL-INDUCED LIVER APOPTOSIS.

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Apoptosis is critically involved in the hepatic pathogenesis induced by acute alcohol exposure. This study was undertaken to test the hypothesis that zinc interferes with apoptotic signaling pathways in the liver leading to inhibition of ethanol-induced apoptosis. Male 129/Sv^{PC}J mice were injected subcutaneously with $ZnSO_4$ (5 mg/kg) in 12 h intervals for 24 h, prior to intragastric administration of ethanol (5 g/kg) in 12 h intervals for 36 h, which has been shown to represent binge drinking in humans. Ethanol-induced apoptosis in the liver was detected by a terminal deoxynucleotidyl transferase nick-end labeling assay and further confirmed by electron microscopy. The number of apoptotic cells in the zinc-pretreated livers were significantly decreased, being only 15 % of that found in the animals treated with ethanol only. Characteristic apoptotic morphological changes observed by electron microscopy were also inhibited by zinc. Importantly, zinc inhibited ethanol-induced activation of caspase-3, the primary executioner protease responsible for alcohol-induced liver apoptosis, as determined by enzymatic assay and Western blotting analysis. Immunohistochemical analysis revealed that zinc inhibited ethanol-induced endogenous hepatocyte Fas ligand activation, which is a key component in signaling pathways leading to hepatic caspase-3 activation and apoptosis. Further studies showed that zinc suppressed ethanol-induced Fas ligand protein expression, as determined by Western blotting, in the liver. These results thus demonstrate that zinc is a potent inhibitor of ethanol-induced liver apoptosis and that this inhibition occurs primarily through zinc interference with Fas/Fas ligand signaling pathway.

581 DIFFERENTIAL INFLUENCE OF STREPTOZOTOCIN (STZ)-INDUCED HYPERGLYCEMIA ON ACETAMINOPHEN (AAP)-MEDIATED APOPTOTIC CELL DEATH AND EXPRESSION OF CYTOKINES IN THE LIVER.

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The diabetic condition and toxic doses of AAP are known to increase CYP2E1 levels, oxidative stress, and promote apoptotic cell death in the liver in addition to modulating cytokine levels. This study was specifically designed to investigate whether diabetic hyperglycemic state, potentiates or antagonizes AAP-induced: i) hepatotoxicity and genomic DNA fragmentation, ii) oxidative stress, iii) cytokine expression, and iv) apoptotic cell death. Hyperglycemia and liver injury were induced by i.p injections of STZ (100mg/kg/day for 8 days) and AAP (300 and 400 mg/kg for 24 hrs) respectively. Male ICR mice were administered Vehicle, STZ, AAP, or STZ+AAP. Blood was collected to evaluate glucose levels and serum chemistry, and livers for histopathology, analysis of DNA integrity, and cytokine levels. Surprisingly, results indicate that STZ-induced hyperglycemia potentiated liver DNA fragmentation, but antagonized hepatotoxicity and oxidative stress. Serum ALT activities were 3.4 and a 7.8 fold lower in STZ+AAP groups compared to AAP alone controls. Oxidative stress markers, lipid peroxidation and total glutathione contents, mirrored the serum chemistry changes. In contrast, the diabetic condition increased DNA fragmentation (Con: 100%; STZ: 206%) coupled with expressions of TNF-alpha (Con: 100%; STZ: 211%) and INF-gamma (Con: 100%; STZ: 205%). AAP exposure to diabetics did not alter cytokine levels but dramatically enhanced the AAP's potential to cause DNA fragmentation (AAP400: 302%; STZ+AAP400:542% of controls values). It appears that diabetes increases hepatocellular antioxidant status and antagonizes AAP-induced hepatotoxicity and oxidative stress but fails to interfere with DNA fragmentation-related events. Induction of CYP2E1 in diabetics may not be universal, and failure of a cause-and-effect relationship between oxidative stress and apoptotic death in the liver may be due to hyperglycemia or breakdown of signaling pathways.

582 MECHANISTIC TOXICOLOGY OF HYDRAZINIUM NITRATE IN PRIMARY RAT HEPATOCYTES.

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Hydrazine (N2H4), a simple diamine and powerful reducing agent, has been used by the US Air Force as a fuel in aircraft and propellant in rockets. Hydrazinium nitrate (HZN), a nonvolatile form of hydrazine, is being examined as a potential replacement for hydrazine, a known hepatotoxicant. The current study was undertaken to (1) evaluate the toxicity of HZN in primary rat hepatocytes *in vitro*, and (2) investigate whether HZN toxicity is mediated by oxidative stress and/or apoptosis. The effects of short-term exposure of hepatocytes to HZN were investigated with reference to viability, biomarkers of oxidative stress and apoptosis. Viability data showed an increase in lactate dehydrogenase (LDH) leakage, and a decrease in mitochondrial activity with increasing concentration of HZN. Studies of oxidative stress biomarkers showed a depletion of reduced glutathione (GSH) and an increase in oxidized glutathione (GSSG), increased reactive oxygen species (ROS) generation, lipid peroxidation and reduced catalase activity. However, there were no changes in glutathione peroxidase and glutathione reductase activities. Depletion of GSH and catalase activity in hepatocytes by buthionine sulfoximine (BSO) and amino triazole, respectively, prior to exposure to HZN increased toxicity. It is obvious from the results obtained that HZN induced biochemical markers of oxidative stress. Further, studies of apoptosis revealed no significant change in total caspase activity, marginal increase in annexin V binding and partial evidence of DNA fragmentation in HZN treated cells. There is a decrease in mitochondrial membrane potential in HZN treated hepatocytes. In summary, the data suggest that short-term exposure to HZN is toxic to primary hepatocytes and its toxicity is attributable to oxidative stress.

583 ASSESSMENT OF APO2L/TRAIL HEPATOTOXICITY *IN VITRO*.

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Recombinant soluble Apo2L/TRAIL induces apoptosis in a wide range of human cancer cell lines while sparing many types of normal cells and is currently being investigated as a candidate for cancer therapy. A polyhistidine-tagged version of Apo2L/TRAIL (Apo2L/TRAIL.HIS) was recently reported to induce substantial apoptosis in primary human hepatocytes, but not in rodent or monkey hepatocytes. To determine if clinical grade, non-tagged Apo2L/TRAIL exhibited activity similar to Apo2L/TRAIL.HIS, the induction of cell death by these versions was investigated in primary human and monkey hepatocytes using the MTT, Alamar Blue™ and Crystal Violet colorimetric assays. Hepatocytes were treated overnight with media, vehicle, Apo2L/TRAIL, Apo2L/TRAIL.HIS (0.01-10 mg/ml), or FasL (a hepatotoxicant serving as a positive control), followed by incubation with MTT, Alamar Blue™, or Crystal Violet. Using all three assays, Apo2L/TRAIL.HIS and FasL induced a marked decrease in cell survival in human hepatocytes, even at concentrations as low as 0.15 mg/ml, whereas Apo2L/TRAIL induced minimal cytotoxicity, even at concentrations as high as 10 mg/ml. While the sensitivity of the Alamar Blue™ and MTT assays was sufficient to detect decreased cell survival induced by FasL, neither assay was adequate for detecting cell death by

Apo2L/TRAIL or Apo2L/TRAIL.HIS in monkey hepatocytes. Monkey hepatocytes treated with Apo2L/TRAIL.HIS, however, showed substantial apoptotic morphology, while Apo2L/TRAIL induced only marginal morphological changes of apoptosis. Additionally, analysis of cell viability using Crystal Violet produced similar findings, with Apo2L/TRAIL.HIS inducing a moderate decrease in cell survival and Apo2L/TRAIL having little effect. The Alamar Blue™ and MTT assays are based on metabolic activity, and their inability to detect cell death in monkey hepatocytes perhaps explains why monkey hepatocytes were resistant to Apo2L/TRAIL.HIS in a previous study using only the MTT assay. Further nonclinical studies are ongoing to evaluate the safety of Apo2L/TRAIL.

584 THE NMDA ANTAGONIST MK-801 POTENTIATES APOPTOSIS IN THE NEONATAL MOUSE BRAIN, LEADING TO HYPERACTIVITY AND LEARNING DISABILITIES IN ADULT MICE.

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It is known that drugs blocking a subtype of glutamate receptors (NMDA) potentiate the natural programmed cell death (apoptosis) occurring during the synaptogenes which can affect learning and memory processes in animals. In humans, this period cover the last three months of pregnancy and first year after birth, which correspond to the first three to four weeks postnatally in rodents. Massive apoptosis during this sensitive period can induce neuropsychiatric disorders like ADHD (attention deficit hyperactivity disorder) in humans. Also, ethanol exposure during pregnancy can manifest as Fetal Alcohol Syndrome (FAS) with characteristic facial malformations, intellectual handicap and psychiatric disorders. In the present study 10-day-old mice were given the NMDA antagonist (+)-MK-801 0.5 mg/kg s.c. three times with 8 hours interval between the doses. One group of mice were killed 24 hours after the first injection and 10 µm thick brain sections were cut and stained using the Fluoro-Jade method. A second group of mice were testet at an adult age of 3 months for spontaneous- and d-amphetamine induced motor activity performance and learning ability in radial arm maze and Morris swim maze. The Fluoro-Jade staining showed a potentiated extensive hippocampal neurodegeneration, but also to a lesser extent in frontal cortex and cerebellum of MK-801 treated animals. Neonatal MK-801 treated mice showed derangement in spontaneous motor activity (hyperactivity) and spatial learning ability. These effects could be reversed when given a low dose of d-amphetamine. Further research should be addressed to investigate anesthetic drugs used in pediatric surgery and child deliveries (ketamine and laughing gas), in order to make a proper risk assessment, regarding dose/effect relationship and possible drug interactions.

585 PCBs ALTER APOPTOSIS IN PRIMARY CULTURES OF HIPPOCAMPAL BUT NOT CORTICAL NEURONS.

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Perinatal exposure to PCBs has been linked to cognitive dysfunction, however, the mechanism(s) underlying this effect are not known. During normal brain development, apoptosis occurs in a region and time specific manner and alteration of normal patterns of apoptosis are associated with deficits in neural function. Therefore, we investigated the possibility that PCBs alter apoptosis in neuronal cell types critical to cognitive function. Primary cultures of cortical and hippocampal neurons were treated for 48 hr beginning on day 5 *in vitro* with varying concentrations of Aroclor 1254 (0.1 – 10 uM), and apoptosis was assessed using the Hoechst stain. Aroclor 1254 (10 uM) significantly increased the percentage of apoptotic cells in hippocampal but not cortical cultures, suggesting that hippocampal neurons are more vulnerable to PCB induced apoptosis. To examine the role of the arylhydrocarbon receptor (AhR) in PCB induced apoptosis, we compared effects in hippocampal and cortical cultures treated with varying concentrations (0.01 to 1 uM) of either PCB 47 or 77, which bind the AhR with low and high affinity, respectively. PCB 47 (1 uM), but not PCB 77, increased apoptosis in hippocampal neurons; neither congener altered apoptosis in cortical cultures. These data suggest that the AhR does not mediate the effects of PCBs on apoptosis. Further studies indicated that the pan caspase inhibitor z-VAD-FMK (60 uM) inhibited increased apoptosis in hippocampal cultures exposed to Aroclor 1254 and PCB 47. However, fluorometric analysis of caspase-3 enzymatic activity indicates that caspase-3 is not activated in hippocampal neurons following PCB exposure, suggesting that caspases or proteases other than caspase-3 mediate PCB induced apoptosis. In summary, our data suggest that alterations in regional profiles of apoptosis subsequent to PCB exposure may be an important mechanism underlying PCB effects on cognitive development.

586 MITOCHONDRIAL EVENTS IN 6-HYDROXY-DOPAMINE-INDUCED APOPTOSIS: IMPLICATIONS FOR PARKINSON'S DISEASE.

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by a loss of dopaminergic neurons in the substantia nigra pars compacta. 6-Hydroxy-dopamine (6-OHDA) is a neurotoxicant that mimics nigral degeneration *in vitro* and *in vivo*. The purpose of this study was to characterize mitochondrial changes occurring during 6-OHDA toxicity. Human neuroblastoma SH SY5Y cells were exposed to 6-OHDA (0-100 µM) which caused time and dose-dependent apoptotic death. After 2, 4, and 7 h exposure to 100 µM 6-OHDA, apoptosis was 3 ± 0.9%, 15 ± 4.0%, and 30 ± 2.5%, respectively, as assessed by Annexin-V staining. Loss of viability of untreated cells was 0.5 ± 0.2% after 7 h. Annexin-V staining paralleled caspase-3 activation, measured by cleavage of the fluorogenic caspase-3 substrate Ac-DEVD-AFC. To investigate the temporal relationship between changes of mitochondrial membrane potential and cytochrome *c* release, SH SY5Y cells were transiently transfected with cDNA cytochrome *c*-GFP and loaded with membrane potential-indicating fluorophore, tetramethylrhodamine methylester (TMRM, 250 nM). Confocal microscopy revealed a 30 ± 6.5% and 97 ± 1.7% decrease in TMRM fluorescence 2 and 4 h after 6-OHDA compared to untreated cells. The pattern of cytochrome *c*-GFP fluorescence was not changed after 2 h exposure to 6-OHDA, but after 4 and 6 h, the percentage of diffuse cells (cytochrome *c* redistribution from mitochondria to the cytosol) was 10% and 48%, respectively. Similar results were obtained by immunocytochemical analysis of native cytochrome *c*. These results indicate that mitochondrial depolarization is an early event in 6-OHDA-induced apoptotic death in SH SY5Y cells. Furthermore, release of proapoptotic cytochrome *c* release is likely the consequence, not the cause, of mitochondrial depolarization. Thus, manipulations to prevent mitochondrial dysfunction may protect against 6-OHDA-induced death in dopaminergic neuroblastoma cells. Supported by NS39469.

587 DIFFERENTIAL ROLE OF CELLULAR FACTORS IN THE MEDIATION OF CYANIDE INDUCED NECROTIC OR APOPTOTIC DEATH.

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Cyanide, a well known neurotoxicant, damages neurons by several mechanisms including peroxide formation, elevation of intracellular calcium and blockade of oxidative metabolism. Cyanide at the concentration range of 100-400µM induced apoptotic cell death in primary cultured cortical neurons characterized by positive TUNEL staining. After the same dose of cyanide, mesencephalic (MC) cell cultures underwent necrosis as confirmed by propidium iodide staining. It was observed that generation of intracellular oxidant species and alterations in mitochondrial functions initiate both modes of cell death. A number of intracellular factors such as caspases, Bcl-2 family members and mitochondrial pro-apoptotic factors play a role in regulation of cellular responses to the toxic insult. Initial studies show that cyanide (100-400µM) in cortical cells resulted in a significant increase in caspase activity and increased cytosolic cytochrome c. No significant changes were observed in nitric oxide synthase (NOS) or superoxide dismutase (SOD) activity. Prior treatment of cells with Z-VAD (non specific caspase inhibitor) inhibited the cyanide-induced increase in caspase activity as well as the apoptosis, suggesting that caspase mediates the cell death in cortical cells. On the other hand MC cells responded with an increased NOS activity and a significant reduction in SOD activity. Pretreatment with L-NAME (NOS inhibitor) greatly reduced cyanide-induced cell death. Thus nitric oxide appears to play a significant role in cyanide-induced oxidative stress in MC cells, but not in cortical cells. Furthermore the anti-cell death factor Bcl-2 was over expressed in MC cells after cyanide treatment suggesting a role for this protein in cyanide induced necrosis. Increased levels of nitric oxide and excess Bcl-2 may favor cyanide-induced necrosis in MC cells whereas cortical cell apoptosis caused by cyanide may involve a mitochondrial pathway. (Supported by NIH grant 04140).

588 CYANIDE-INDUCED REACTIVE OXYGEN SPECIES GENERATION AND ELEVATED CYTOSOLIC CALCIUM UP-REGULATE CYCLOOXYGENASE-2 EXPRESSION IN CORTICAL CELLS.

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The role of cyanide-induced reactive oxygen species (ROS) generation in cyclooxygense-2 (COX-2) expression is unclear. COX-2 is an inducible enzyme involved in arachidonic acid metabolism, which generates ROS as side products. It has been shown that ROS up-regulates COX-2 expression. In the present study, the role of

ROS generation in cyanide-induced up-regulation of COX-2 and apoptosis was determined. After treatment with cyanide (50-300μM) there was a prompt increase of intracellular ROS generation. Inducible COX-2 expression followed ROS generation, showing that ROS regulate COX-2 expression. Pretreatment of cells with antioxidants (PBN, SOD or CAT) inhibited cyanide-induced COX-2 expression as shown by western blotting and RT-PCR. Direct stimulation of cells with H_2O_2(100-200μM) for 4h induced a dose-dependent increase of COX-2 and apoptosis (24h) similar to cyanide. Cyclosporine A, a specific blocker of mitochondrial membrane potential and permeability transition, blocked cyanide-induced ROS generation and COX-2 expression. When cyclosporine A was added at 30 min after cyanide incubation, the effect on COX-2 expression and apoptosis was abolished. Measurement of PGE_2, a metabolite of COX-2, showed that pretreatment with antioxidants (PBN, SOD or CAT) reduced cyanide-induced COX-2 activity. When added at 30 min after cyanide, antioxidants still inhibited neuronal apoptosis, indicating that ROS generation from COX-2 metabolism plays a key role in the apoptosis. The results from western blot and RT-PCR showed that Ca^{2+} is a mediator in cyanide-induced COX-2 up-regulation. Cyanide did not increase COX-2 expression when cells were incubated with cyanide in the Ca-free medium. BAPTA, a membrane permeable chelator of Ca, blocked cyanide-induced COX-2 expression and apoptosis. These results show that cyanide increases intracellular ROS and free-Ca^{2+} to mediate up-regulation of COX-2 and the sequent ROS evolving from COX-2 metabolism play a role in cyanide-induced apoptosis. (Supported by NIH grant ES 04140)

589 THE ROLE OF CASPASE III INHIBITION IN METHAMPHETAMINE-INDUCED ALTERATIONS IN P53 AND *BCL-2* EXPRESSION: CORRELATION WITH DOPAMINERGIC NEUROTOXICITY.

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Methamphetamine (METH) is a widely used drug of abuse. It causes long-lasting reductions in dopamine (DA) content, tyrosine hydroxylase activity and dopamine transporters in the striatum. It also produces oxidative stress by generating reactive oxygen and nitrogen species. METH has been reported to upregulate p53 and downregulate *bcl-2* expression in striatum of mice after repeated doses. Caspase-3 is a downstream effector involved in the dissolution of the nucleus. Activation of Caspase-3 follows the onset of apoptosis. In the present study, we studied the role of caspase inhibition on METH-induced alterations in the expression of striatal p53 and *bcl-2* in mice. Adult male C57 mice (n=8/group) were treated with 4x10 mg/kg of METH at 2 h intervals. Another groups of mice also received a specific Caspase-3 inhibitor (CIII, 0.5 mg/kg) or a general caspase inhibitor (GC, 0.5 mg/kg) along with METH or alone. Saline treated animals served as control. Animals were sacrificed 72 h after the last dose of METH. METH administration resulted in significant up-regulation of p53 and down-regulation of *bcl-2* in mice striatum. GC failed to protect against this genetic alteration caused by METH but CIII significantly protected against METH-induced genetic changes in the mice striatum. METH also resulted in the significant depletion of DA and its metabolites DOPAC and HVA. CIII provided a significant attenuation whereas GC failed to provide any protection against dopaminergic neurotoxicity. Therefore, the present data demonstrated that METH-induced dopaminergic neurotoxicity caused genetic alterations, which can lead to the onset of programmed cell death, however, it can be protected by specifically targeting the downstream effector action of Caspase-3.

590 ROLE OF MITOCHONDRIAL BCL-2 PROTEIN AND DOPAMINE IN ENVIRONMENTAL CHEMICALS-INDUCED DOPAMINERGIC CELL DEATH.

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Several epidemiological studies have implicated chronic exposure to certain pesticides and metals in the etiology of Parkinson's disease. Manganese and dieldrin are two prominent environmental toxicants that have been associated with Parkinson's disease. We previously demonstrated that dieldrin and methylcyclopentadienyl manganese tricarbonyl (MMT) share similar cell death signaling process and induce apoptosis in dopaminergic cells. In the present study, we have further characterized biochemical mechanisms underlying dieldrin- or MMT-induced selective dopaminergic toxicity. When exposed acutely, both dieldrin and MMT show relatively selective cytotoxicity to dopaminergic PC12 cells with EC_{50} at 142μM and 200μM, respectively. EC_{50} of dieldrin in HCN-2 cells (human cortical neuronal cells) and α-TC (pancreatic B cells) is 292μM and 351μM, respectively, and EC_{50} of MMT in striatal GABAergic cells is 577μM. The dieldrin- or MMT-induced generation of reactive oxygen species (ROS) and apoptotic cell death are significantly attenuated by pre-treatment with α-methyl-*p*-tyrosine or deprenyl, indicating that the presence of dopamine may, in part, contribute to susceptibility of dopaminergic cells to these environmental neurotoxicants. Furthermore, using Bcl-2 transfected PC12 cells, we have demonstrated that these chemicals may target mitochondria and initiate apoptotic cell death signaling cascades. Reduction of mitochondrial membrane potential, caspase-3 activation and DNA fragmentation are significantly ($p<0.01$) attenuated in Bcl-2 overexpressed PC12 cells following acute exposure to dieldrin (10-100μM) or MMT (10-200μM). Together, our results suggest that excessive dopamine release and mitochondrial dysfunction may serve as intrinsic susceptibility factors in environmental factor-induced dopaminergic degeneration. Supported by NIH grant ES10586

591 ACTIVATION OF CASPASE-3 BY DIETHYLDITHIOCARBAMATE (DDC) IN RAT HIPPOCAMPAL ASTROCYTES WITH OR WITHOUT GLUTATHIONE.

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Diethyldithiocarbamate (DDC), is a carbamate derivative and potent metal chelator. It has been shown to bind and redistribute copper in a lipophilic complex to many organs including the central nervous system. We have previously demonstrated by TUNEL and DNA laddering techniques that 35μg/ml and 350μg/ml DDC induced apoptosis or programmed cell death in rat hippocampal astrocytes treated for 1hr. Caspases, also known as interleukin-1-β-converting enzyme (ICE), are cysteine proteases that have been implicated in apoptosis. The activation of the caspase cascade requires the activation of initiator caspases by a toxic insult which in turn activates effector caspases. Caspase-3 cleaves a set of vital proteins and executes the cascade of events leading to the apoptotic degradation phase. Glutathione plays a major role in the antioxidant defense system. It has been documented to participate in the detoxification of xenobiotics. It has also been documented in the literature that glutathione maintains cytoskeletal integrity by stabilizing intracellular sulfhydryl status. Previous experiments have shown that glutathione (10mM) post-treatment protects against DDC insult. The purpose of this experiment was to determine whether caspase-3 may be induced following 1hr treatment with DDC and whether glutathione prevents caspase-3 activation. Rat hippocampal astrocytes grown in Dulbecco's modified Eagle's essential media were treated with 35 and 350 μg/ml DDC at subconfluency for 1 hr. Astrocytes were then left to recover with or without 10mM glutathione in DMEM for 48 h at which time caspase-3 activity was assayed. Preliminary studies suggest that caspase-3 is activated in astrocytes treated with 35 and 350 μg/ml DDC. Glutathione post-treatment prevents the activation of caspase-3 in both treated groups.

592 CASPASE-3 MEDIATED PROTEOLYTIC CLEAVAGE OF PKCδ PLAYS A CRITICAL ROLE IN METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL (MMT)-INDUCED APOPTOTIC CELL DEATH IN MESENCEPHALIC CLONAL CELLS.

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The cellular mechanisms underlying selective dopaminergic degeneration in PD remains elusive. Oxidative stress has been suggested to contribute the pathogenesis of PD. Additionally, environmental exposure to certain pesticides and transition metals such as manganese have a positive association with increased incidences of PD. We characterized oxidative stress dependent cellular events by exposing an immortalized rat mesencephalic dopaminergic cell line ($1RB_3AN_{27}$) to an organic form of manganese compound, methylcyclopentadienyl manganese tricarbonyl (MMT). Expsoure of MMT (30-100 μM) resulted in a rapid increase in generation of reactive oxygen species (ROS) within 5-15 min and was followed by release of mitochondrial cytochrome C into cytoplasm. Cytochrome C then activated a proapoptotic cysteine protease, caspase-3 (6-fold) in a time- and dose-dependent manner. Interestingly, we also found that MMT exposure induces a time- and dose-dependent 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 MMT-induced proteolytic cleavage of PKCδ, indicating that cleavage is mediated by caspase-3. Attenuation of ROS generation, caspase-3 activation and PKCδ activity prior to MMT treatment almost completely suppressed DNA fragmentation. Additionally, overexpression of catalytically inactive $PKC\delta^{K376R}$ (dominant negative mutant)prevented MMT-induced apoptosis in immortalized mesencephalic dopaminergic cells. Preliminary data in *ex vivo* rat brain slices exposed to MMT indicated proteolytic cleavage of PKCδ within 3 hr of treatment. For the first time, these data demonstrate that caspase-3 dependent proteolytic activation of PKCδplays a key role in oxidative stress-mediated apoptosis in dopaminergic cells following exposure to an environmental neurotoxic agent.

593 METHYLMERCURY INTERACTS WITH MUSCARINIC RECEPTORS DURING NEURONAL DEATH IN RAT CEREBELLAR GRANULE CELLS.

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Cerebellar granule cells are highly susceptible to MeHg-induced neuronal death both *in vivo* and *in vitro*. One hallmark of this neuronal death is severe Ca^{2+} deregulation, which contributes to delayed neuronal death in primary cultures of rat cerebellar granule neurons. Within granule cells, the SER represents a high affinity, low capacity Ca^{2+} store which may contribute to MeHg-induced Ca^{2+} elevations by release of Ca^{2+} though inositol-1, 4, 5-phosphate (IP_3) receptors. Because this Ca^{2+} can be released through activation of M3 muscarinic acetylcholine receptors, we examined whether inhibition of these receptors is cytoprotective during MeHg exposure. We used neonatal rat cerebellar granule neurons in primary culture to measure cell viability using a calcein-ethidium homodimer live/dead cytotoxicity assay. Previous results indicated that down-regulation of muscarinic and IP_3 receptors with 24 hr bethanechol (1 mM) was neuroprotective (Martinez *et al., The Toxicologist* 60:184, 2001), suggesting a role for muscarinic and/or IP_3 receptors in MeHg-induced cell death. In our experiments, the protection afforded by bethanechol was not affected by the nicotinic receptor antagonist dihydro-β-erythroidine, indicating that protection was through the muscarinic receptors. However, application of atropine alone (10 μM) did not attenuate cell death caused by 0.2-1.0 μM MeHg. Inhibition of Ca^{2+} release through the IP_3 receptor with 1 μM xestospongin C was also not protective. Interestingly, 24 hr application of 1 μM xestospongin C or acute application of the phospholipase inhibitor U73122 (1 μM) alone caused significant cell death, highlighting the importance of SER Ca^{2+} signaling pathways in maintenance of cell viability. These results suggest that the protection afforded by 24 hr BCh treatment occurs through muscarinic receptors, and that disruption of this pathway is neurotoxic in cerebellar granule neurons. Supported by NIEHS grants R01 ES03299 and T32 ES07255.

594 THE 2-NITROIMIDAZOLE RADIOSENSITIZER CI-1010 INDUCES SELECTIVE APOPTOSIS IN THE PHOTORECEPTORS OF C57BL/6 MICE.

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CI-1010, a bifunctional nitroimidazole, produces selective apoptosis in photoreceptors *in vivo*. CI-1010 triggers opening of the mitochondrial permeability transition (MPT) pore in SY5Y neuroblastoma cells following oxidation of the intracellular space. This study investigates *in vivo* mechanisms of CI-1010-induced retinal toxicity. Adult C57BL6 mice treated with a single intraperitoneal injection (1000 umol/kg) were necropsied at 4, 12, 24, 48, 72, and 96h following treatment. Morphological evaluation by light microscopy reveals retinal degeneration by 96h localized to the photoreceptor (PRL) and outer nuclear layer (ONL) of the retina. By 12h, the PRL appears vacuolated, compressed, and poorly organized. The pattern of retinal degeneration is time-dependent and characterized by thinning of both retinal layers due to loss of photoreceptor segments and nuclei, culminating in the collapse of the PRL. Apoptotic bodies are visible in the ONL by 72h and many nuclei label with (TdT) mediated 3'-OH fluorescein end-labeling. Western blot analysis of retinal homogenates from treated mice show decreased expression of the anti-apoptotic protein, Bcl-xL, at 4h, remaining less than control at all timepoints. Bcl-xL is responsible for stabilization of the MPT pore in the closed position. Reduced Bcl-xL expression may destabilize the MPT pore in the PRL. Analysis of caspase 9 and 8 activation suggests involvement of both the mitochondrial and non-mitochondrial pathways by 24 h. Caspase 3 is active at 72h. The precise role of mitochondria and caspase activation in CI-1010-induced neuronal cell death is unknown. However, these results suggest that retinal apoptosis induced by CI-1010 is mediated by caspase-3 activation subsequent to the execution of a mitochondrial pathway of cell death.

595 BCL-XL INHIBITS LEAD-INDUCED ROD PHOTORECEPTOR APOPTOSIS BY BLOCKING THE INCREASE IN MITOCHONDRIAL CONTACT SITES.

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Photoreceptor apoptosis and visual deficits occur in man and animals with inherited and chemical-induced retinal degenerations, and retinal diseases and injuries. Rod-selective apoptosis is produced by developmental lead exposure in mice and by *in vitro* lead and/or calcium: the latter activates the cytochrome c-caspase cascade of apoptosis (JBC, 2000; SOT, 2001). This study used wild-type and transgenic mice overexpressing Bcl-xL in photoreceptors to examine the role of mitochondria in lead-induced rod apoptosis. Newborn mice were raised by dams drinking water (controls) or 0.15% lead acetate solution. At weaning (BPb = 26 μg%) studies of rod mitochondria were conducted in all four groups using three-dimensional electron microscopic tomography, JC-1 staining, immunoblots for cytochrome (cyt) c, caspase assays and oxygen consumption. There were no differences between the four treatment groups for 5 of 7 different measurements of rod mitochondrial substructures (crista widths, crista junction diameters, outer (OMM)-inner membrane widths, contact site widths, contact site diameters) and no physical disruptions of the OMM or swollen mitochondrial matrices. Lead-induced rod apoptosis was accompanied by calcium overload, increases in mitochondrial contact site area and density, decreases in rod mitochondrial respiration and membrane potential (depolarization), cyt c release and caspase activation. Bcl-xL overexpression completely blocked the lead-induced increase in contact sites and all apoptotic events, except calcium overload, and maintained normal rod respiration. These results suggest that Bcl-xL is located and functions at mitochondrial contact sites to block the opening of the permeability transition pore. These findings have relevance for therapies in human and animal retinal and neuronal degenerations where lead exposure, calcium overload and/or mitochondrial dysfunction occur. Supported by NIH Grants ES03183, EY07088, RR04050, NS14718, KD54441.

596 MERCURIC CHLORIDE-INDUCED PROGRAMMED CELL DEATH IN MOUSE LEYDIG CELL LINE (LFG6).

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There is strong evidence that exposure to heavy metals, in general, and mercurial compounds in particular, impair the function of the male reproductive system. A multigenerational study, recently, completed our laboratory, demonstrated that mercuric chloride causes a reduction of fertility indices, reproductive indices and testosterone level. Recent studies have demonstrated that germ cell apoptosis increases after testosterone withdrawal from the testes. The present study was undertaken to determine whether testosterone reduction associated with exposure to mercuric chloride occurred due to Leydig cells death by the apoptosis. Mouse Leydig cell line (LFG6) was exposed in Dulbecco's modified Eagle's medium (DMEM) to mercuric chloride concentrations 0.0, 3.75, 7.5, 15 or 30 μM. Viability of Leydig cell was tested using trypan-blue exclusion assay after 1, 6, 12 and 24 hours of mercuric chloride exposure. Cells in control, 2.75 and 7.5 μM groups had a viability of approximately 96%, regardless of exposure time. In contrast, the viability of the cells in15 and 30 μM treatments gradually declined to approximately 85% and 60 respectively, after 24 hours of exposure. Agarose gel electrophoresis and ligation-mediated polymerase chain reaction (LM-PCR) were used to assess the presence of apoptosis. The nucleosomal DNA fragmentation was quantitated visually and by using Fluor-S Multilmager. The intensity of DNA ladder in exposed cells increased significantly in a concentration- and- time dependent manner when compared to that of the controls. These results suggested that mercuric chloride caused a significant apoptosis in a Leydig cell line and the mechanism of mercuric chloride -induced testosterone level reduction *in vivo* may be related to the induction of apoptosis.

597 EVIDENCE OF MITOCHONDRIA-ASSOCIATED SIGNALING IN TESTICULAR GERM CELL APOPTOSIS IN ADULT C57BL/6(B6) MICE AND SPRAGUE-DAWLEY RATS EXPOSED TO MONO-(2-ETHYLHEXYL) PHTHALATE (MEHP).

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The Fas signaling pathway has been previously characterized as a key mediator of germ cell apoptosis, particularly after toxicant-induced Sertoli cell injury. Recent reports have indicated that cell-damaging agents can stimulate apoptosis through a "stress-induced" mitochondria-regulated signaling pathway. In stress-induced apoptosis, the release of cytochrome C (initiated by the pro-apoptotic proteins Bak and Bax) appears to be a critical event. Cytochrome C release results in the activation of caspase-9 and eventual cell death. Interestingly, caspase-8 activation has been shown to increase the release of cytochrome C by truncating Bid to tBid, which can then oligomerize with Bax or Bak causing the release of cytochrome C. We propose that the mitochondria-associated signaling system is important in the initiation of apoptosis after MEHP-induced germ cell injury and may act in conjunction with the Fas signaling system. Adult C57 mice and adult Sprague-Dawley rats were exposed to an acute dose of MEHP (1 g/kg p.o.). Western blot analyses demonstrated early increases (by 3 h post-MEHP exposure) in cytosolic cytochrome C and subsequent increases in active caspase-9 and caspase-3 in mice. Immunohistochemisty (IHC) on both frozen tissue sections and fresh testis touch

preparations revealed germ cell-specific protein expression and/or localization of Bax and caspases in rat testes. These immediate changes of stress-related signaling proteins occurred simultaneously with earlier observed increases in death receptors (e.g., Fas, death receptor 4, etc.) and caspase-8, suggesting a link or possibly cooperation between death receptor- and mitochondria-regulated apoptosis signaling pathways. (Supported by R01ES09145, Training Grant ES07247, NIEHS Center Grant 07784).

598 PULSATILLA NIGRICANS (HOMEOPATHIC DRUG) TREATMENT INCREASED APOPTOSIS AND ATRESIA IN RAT OVARIAN FOLLICLES ALONG WITH DECREASED DNA LEVELS.

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Pulsatilla nigricans is an age-old homeopathic drug prescribed for various reproductive ailments especially in women. The drug contains an alkaloid annemonin, annemonic acid and potassium sulphate. The mode of action of the drug and the effect of different doses (Potencies) is not well understood. Hence the present study was under taken to evaluate the effect of Pulsatilla nigricans on paracrine mechanisms involving follicular atresia along with quantification of DNA. Female albino rats of wistar strain were administered with different potencies of the drug orally on alternate days for a period of four weeks. The biochemical study revealed that 200c potency has shown a significant decline in the DNA content of the ovary. This was indicative of low rate of protein synthesis and steroidogenesis especially in the ovary. Likewise histological studies also revealed consistent follicular atresia with prolonged diestrous and metaestrous phases when treated with 200c potency. The drug with 200c potency had shown a maximum decline in the DNA levels possibly because of increased DNA fragmentation when compared with the other potencies. The same potency showed maximum follicular atresia and significant number of apoptotic cells ($P < 0.005$). Ovarian cycle in general involves a complex networking of tissue growth and differentiation of programmed cell death / atresia. In conclusion, our findings suggested that 200c potency of Pulsatilla is a strong antifertility compound, which can be used as a safe and cheaper contraceptive in the developing countries.

598A DAMAGE OF DNA IN CULTURED SKIN CELLS BY LOW DOSES OF AS(III).

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We have previously established the higher toxicity of As(III) than that of As(V) for nucleated cells including skin cells on integrity of the cell membrane, the rate of DNA, RNA and protein synthesis in primary human skin cultures. In this study we demonstrated that: a) exposure of human keratinocytes (HK) cultures to As(III) produces a dose-dependent decrease in protein concentration and shows a significant decrease at 500µg/L (over 30% of control) and 1000 µg/L (over 45% of control) within 48 hrs of exposure; b) transition of apoptotic changes to necrotic damage in artificial skin topically exposed to As(III) as determined by *in situ* DNA fragmentation analysis at the single cell level using the TdT-mediated biotin dUTP nick end-labelling (TUNEL assay); c) low doses (5-100µg/L) of As(III) cause single strand DNA breaks and alkali labile dose and time dependent damage in HK and fibroblasts (HF)). Exposure of HF to 5µg/L As(III) for 30 min results in an increase of 165% in the comet moment. At 10µg/L this increase is 195% rising to 282% at 100µg/L and 728% at 500µg/L. We used Telomerase PCR ELISA to quantitate telomerase activity in cell extracts from submerged cultures of HF, exposed to As(III). Our experiments reveal that increasing of the exposure dose to100 and 1000µg/L of As(III) from 15 min to 30 min elevated telomerase activity in both cases. The enhancement is greater at the higher (1000µg/L) As(III) concentration (>3 times) and less pronounced at the lower (100µg/L) exposure (~1.6 times) thereby reflecting the significantly greater chromosome damage produced by the higher As(III) doses. The involvement of telomerase in the regulation of cell growth and viability *via* the protection against DNA damage makes it an appropriate target for analysis of the extent of molecular damage at the genomic level produced by As exposure.

599 INDUCTION OF CYP ISOFORMS BY PESTICIDES AND DEET AS MONITORED BY THE BDNA ASSAY.

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Drug-drug interactions often occur when important drug metabolizing enzyme levels are altered as a result of exposure to another drug. As a consequence of this phenomenon, drugs are routinely screened for their ability to induce cytochrome P450 enzymes, one of the more important drug metabolizing enzyme families. Pesticides are also known to induce cytochrome P450 enzymes in humans. Studies of gulf war veterans suggest that pesticide-pesticide or pesticide-drug interactions may have had negative impacts on veterans. Our previous rodent studies confirm that pesticides and insect repellants such as DEET can induce enzymes important in pesticide activation and/or detoxication reactions. To determine possible pesticide interactions in humans, cultured human hepatocytes were exposed to gulf war pesticides and repellant and CYP450 mRNA levels quantified using the branched DNA signal amplification assay. Preliminary results indicate that several CYP isoforms are altered by permethrin, chlorpyrifos, and DEET. The insecticide permethrin significantly induced the CYP isoforms 2A6 and 2B6. Chlorpyrifos was surprisingly efficient in its induction of CYP1A1, 1A2, and 3A4, but also repressed 2E1. The insecticide repellant DEET induced CYP3A4, 2B6, and 2A6. Since our previous studies using human lymphoblast cell lines demonstrated the importance of human CYP2B6 in the activation of chlorpyrifos to chlorpyrifos-oxon, this work suggests that pesticides and repellants such as DEET have the potential to increase and/or decrease pesticide susceptibility.

600 FORMATION OF UBIQUITIN-CYTOCHROME P450 3A CONJUGATES IN ISOLATED MICROSOMES.

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A novel microsome system is described that conjugates CYP3A and ubiquitin to form high molecular weight (HMW) complexes. The induction of this process in isolated microsomes appeared to be dependent upon extended *in vivo* treatment with potent CYP3A inducers. Treatment of the microsomes to remove potential contamination of cytosolic proteins did not prevent the formation of the HMW CYP3A conjugates. Immunoprecipitation of HMW CYP3A and Western blot analysis indicated that this protein was complexed with ubiquitin. Even so, the conjugation reaction did not require addition of ubiquitin, ATP, Mg++ or cytosol and, as such, was unlikely to be dependent upon the ubiquitin activating enzyme E1. Furthermore, mass spectrometric analysis of tryptic peptides strongly suggested that the HMW CYP3A was in molar excess to ubiquitin. This result suggested that the HMW CYP3A may be conjugated to itself or a diffuse pool of already ubiquitinated proteins present in the sample. CYP3A substrate blocked formation of the HMW CYP3A in the incubated microsomes. These results suggest that extended induction of CYP3A expression results in the activation of microsomal factors that catalyzed the formation of HMW CYP3A conjugates. This conjugation reaction may be related to the substrate-dependent stabilization of CYP3A observed *in vivo* but appears to be distinct from the classical ubiquitination pathway.

601 SAFETY EVALUATION OF CHRONIC DIETARY EXPOSURE TO 3, 3-DIINDOLYLMETHANE (DIM) IN SPRAGUE-DAWLEY RATS.

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Indole-3-carbinol (I3C), a naturally occurring component of the human diet, is found in significant concentrations in cruciferous vegetables. In an acidic environment, such as the stomach, I3C forms condensation products, one of the most abundant of these being DIM. DIM is marketed as a dietary supplement but little is known about the safety of long-term exposure. Male and female Sprague-Dawley rats were fed either control diet, diet containing the proposed human dose of Bioresponses' DIM equaling 6.45 mg/kg/day, or diet containing ten times the proposed human dose equaling 64.5 mg/kg/day. The experiment was terminated after 3 or 12 months of exposure. Small animal chemistry panels were performed along with histopathological examination of the liver, kidneys, adrenals, and colon of all animals, the testicles, prostate, and epididymus for the males, and the uterus and overies for the females. The total cytochrome P450 (CYP) content of the liver was determined along with the levels of the specific isoforms CYP1A1 in liver and colon and CYP3A2 in the liver. No significant differences between groups were found in blood chemistry. Most notably no increases were seen in markers for liver or other tissue damage. Tissue examination yielded no observable differences grossly or histologically between groups, although a high number of hyaline casts were found throughout the kidney tissue of all animals. Total hepatic CYP levels were induced (28%) only in males. Slight induction of CYP1A1 and CYP3A2 was seen in liver and CYP1A1 in colon for both sexes. Comparison to I3C indicates that DIM is a markedly less potent inducer of CYP1A1 in the rat. Supported by the OSU Ag. Res. Fdn.

602 EVALUATION OF ALBENDAZOLE SULFOXIDE AS A PROBE FOR CYP1A1 ACTIVITY.

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Albendazole Sulfoxide (ABZSX) is the active metabolite of the anthelmintic drug Albendazole (ABZ). Previous reports on ABZ pharmacokinetics in rats (pretreated with different cytochrome P450 (CYP) inducers) suggest that ABZSX is metabolized to Albendazole Sulfone (ABZSN) by cytochrome P450 1A1 (CYP1A1). In order to test this compound (ABZSX) as a probe to evaluate CYP1A1 activity *in vivo*, three experimental groups were used: a) Control group; b) Induced group; and c) Inhibited group. All experimental groups were treated p.o. with a single dose of Albendazole Sulfoxide (20 mg/kg, suspended in corn oil). Groups b) and c) were previously treated with a CYP1A1 inducer (β-naphtoflavone) and a CYP1A1 inhibitor (α-naphtoflavone), respectively. After treatment with ABZSX, plasma levels of ABZSX and ABZSN were evaluated through 18 h by HPLC. CYP1A1 activity (Ethoxyresorufin *O*-deethylase) was measured in liver microsomes from animals belonging to each experimental group. Correlations between pharmacokinetic parameters and hepatic CYP1A1 activity will be presented.

603 QUININE METABOLISM AS A PROBE FOR THE EVALUATION OF RAT HEPATIC CYP1A1 AND CYP3A.

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The *in vitro* metabolism of quinine was investigated with 15 recombinant rat cytochrome P450 enzymes. It was found that the CYP3A1 enzyme produced 3-OH quinine as the major product (90% of all metabolites detected) of quinine biotransformation. The recombinant CYP3A2 was active in metabolizing quinine into 3-OH quinine but with a rate approx. 30% of that for CYP3A1. No significant formation of 3-OH quinine was found in the experiments with other 13 recombinant rat CYP. Recombinant rat CYP1A1 formed O-desmethylquinine as the major metabolite of quinine with no significant formation of 3-OH quinine. No significant formation of O-desmethylquinine was found in the experiments with either CYP3A1, CYP3A2 or CYP1A2, as well as with other 11 tested recombinant rat CYP. In liver microsomes incubated with quinine, the rates of formation of 3-OH quinine were 0.26, 0.32, 22.0 and 1.0 nm/min/mg protein for female control, MC- and DEX-induced and male control rats, respectively. Among the microsomal preparations tested the only female MC-induced microsomes were active in the production of O-desmethylquinine. Overall, the data obtained with recombinant rat cytochrome P450 enzymes are in the good agreement with the data obtained with hepatic microsomes from variously treated rats. Enzymes kinetics (Vmax, Km and Clint) determined for rat CYP1A1 and CYP3A1 indicate that both enzymes are similarly active in quinine biotransformation although each produces different metabolite. This study indicates that two different products of quinine biotransformation could be used as suitable probes for the *in vitro* and/or *in vivo* evaluation of CYP1A1 and CYP3A metabolic activities. (Supported by GM 44982 and ES 05022)

604 STRUCTURE-ACTIVITY RELATIONSHIPS (SARS) FOR *IN VITRO* INHIBITION OR INDUCTION OF AROMATASE (CYP19) ACTIVITY BY VARIOUS PESTICIDES.

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SARs were assessed for aromatase (which converts androgens to estrogens) inhibition or induction by various pesticides in H295R human adrenocortical carcinoma cells. The effects of 13 pesticides were assessed and compared to literature data for *in vitro* inhibition or induction by structurally similar pesticides. Among the most potent aromatase inhibitors were the fungicides imazalil and prochloraz, which both contain an imidazole and chlorinated aromatic moiety. The structurally similar imidazole fungicides (in order of decreasing potency) tioconazole, clotrimazole, econazole, miconazole and isoconazole have previously been reported to be good inhibitors. Less potent inhibitors in H295R cells were the triazole containing fungicides difenoconazole, penconazole and propiconazole. The structurally similar triazole fungicides triademenol and triadimefon were also reported previously to have similar inhibition potencies. The hydroxy-containing triazoles tebuconazole, hexaconazole and paclobutrazole were almost inactive in H295R cells, as were the pyrimidine fungicides fenarimol (a known microsomal aromatase inhibitor) and nuarimol, probably due to the limited cellular uptake of these relatively more polar compounds. Enzyme kinetic analyses indicated that the imidazole fungicides were uncompetitive-, whereas the triazole fungicides were competitive inhibitors of aromatase with K_i values in the low micromolar range. The triazine herbicides atrazine, simazine and propazine, the structurally similar vinclozolin and to a lesser extent the hydroxylated triazole fungicide diclobutrazole were inducers of aromatase activity. Atrazine and vinclozolin act by increasing intracellular cAMP levels, possible through inhibition of phosphodiesterase activity, suggesting this enzyme as a target for SAR analysis of aromatase inducers in H295R cells. These SARs may be useful to identify chemicals with the potential to disturb the androgen/estrogen balance through an interaction with aromatase.

605 HYPEROXIA DIFFERENTIALLY MODULATES CYTOCHROME P450 EXPRESSION IN NEWBORN AND ADULT RATS: IMPLICATIONS FOR OXYGEN-MEDIATED LUNG INJURY.

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Supplemental oxygen is frequently administered to infants having pulmonary insufficiency. Newborn rats show increased tolerance to hyperoxic lung injury than adult rats. In this study, we tested the hypothesis that exposure of newborn or adult rats to hyperoxia would differentially regulate hepatic and pulmonary cytochrome P450 (CYP) isoforms, a phenomenon that may contribute to the increased tolerance to injury in the neonatal rats. Newborn Fisher rats or adult male rats were maintained in room air or exposed to hyperoxia ([gt] than 95% O2) for 1-7 days, and animals were sacrificed at selected time points. Pulmonary and hepatic CYP1A1, CYP1A2, and CYP4F4 mRNA expression was determined by RT-PCR. All newborn rats survived 7 days of hyperoxic exposure. On the other hand, none of the adult rats survived beyond 60 h of hyperoxia. In the newborn liver, hyperoxia markedly induced CYP1A1, CYP1A2 and CYP4F4 expression over room air controls after 2-4 days of exposure, but the expression of CYP1A1, but not CYP1A2 or 4F4, declined after 7 days. In the newborn lung, hyperoxia induced CYP1A1 and CYP4F4 expression early in the time course (1-2 days), but expression of both isoforms declined by 7 days. In adult rats, the hepatic expression of CYP1A1/1A2 and CYP4F4 was enhanced between 24-48 h of hyperoxia, followed by decline at 60 h. In contrast, pulmonary CYP4F4, was induced by hyperoxia only at 60 h. The sustained induction by hyperoxia of CYP1A2 and CYP4F4 genes in the newborn but not adult liver suggests an important protective role for these enzymes against hyperoxic lung injury. Furthermore, the augmentation of pulmonary CYP4F4 gene expression early in the time course of hyperoxic exposure in the newborn rat may contribute to the increased tolerance to oxygen-induced lung injury. (Supported in part by NIH grant ES09132 and by ALA.)

606 ALLEVIATION OF HYPEROXIC LUNG INJURY IN THE RAT BY THE CYTOCHROME P4501A INDUCER 3-METHYLCHOLANTHRENE.

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Supplemental oxygen administration is used extensively in the treatment of pulmonary insufficiency that is commonly observed in in premature infants. We reported earlier that exposure of rats to hyperoxia for 48 h leads to induction of hepatic CYP1A1/1A2 enzyme activities, followed by decline at 60 h, the time point that coincides with severe respiratory distress and lung damage. In this study we tested the hypothesis that pretreatment of rats with the CYP1A inducer 3-methylcholanthrene (MC) would protect animals from lung damage caused by subsequent hyperoxic exposures. We treated adult male Sprague-Dawley rats with MC (93 μmol/kg) or vehicle only, once daily for 4 days. After 1 or 15 days after MC withdrawal, the animals were either maintained in room air or exposed to hyperoxia (> 95% oxygen) for up to 60 h. Activities of pulmonary and hepatic ethoxyresourufin-O-dethylase, which is specific for CYP1A1, were about 30-40-fold elevated in the MC-treated rats at both 1 and 15 day time points, compared to vehicle-treated controls. Seven out of 9 control rats survived 60 h of hyperoxia. On the contrary, 12/12 MC-treated animals survived 60 h of hyperoxia. This was the case for both the 1 and 15 day time points. Vehicle-treated animals exposed to hyperoxia for 60 h had severe measurable pleural effusions (~ 10.4 ± 2.0 g), which is an index of lung injury. In contrast, MC-treated animals after 60 hyperoxia had significantly lower pleural effusions, being 3.1 ± 1.2 (g) and 6.3 ± 1.7 (g), respectively, for 1 and 15 day time points. The decreased susceptibility of MC-treated animals to oxygen-induced lung damage, in conjunction with the sustained CYP1A1 expression in these animals, supports the hypothesis that CYP1A1 induction plays a beneficial role in hyperoxic lung injury. (Supported in part by NIH grant ES09132 and by ALA.)

607 UROPORPHYRIA CAUSED BY FE AND ALA IN MICE - THRESHOLD REQUIREMENTS FOR FE AND CYP1A2.

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Previous work has implicated both CYP1A2 and Fe in experimental uroporphyrin (URO) accumulation caused by polyhalogenated compounds, 5-aminolevulinate (ALA) and Fe. In mice, strain differences in sensitivity for uroporphyria caused by ALA and Fe have been observed. We examined whether the higher sensitivity to uroporphyria caused by ALA alone in SWR compared to C57BL/6 mice was due to higher endogenous levels of hepatic Fe and CYP1A2 in SWR mice.The ALA and Fe-treated Cyp1a2 (+/-) heterozygote which has CYP1A2 protein levels about 60 % of the Cyp1a2 (+/+) wild-type, accumulated much less URO than the wild-type, whereas Cyp1a2 (-/-) mice accumulated no URO. In C57BL/6 mice treated with ALA, increasing CYP1A2 about 2-fold by a low dose of PCB126, increased URO accumulation by 6-fold, if Fe was also given. A Dose-Response study with Fe dextran in mice given PCB126 and ALA, showed that hepatic levels of Fe greater than 800 microg/g wet weight were required to cause considerable URO accumulation. Perls staining revealed that at low Fe dextran doses, Fe accumulation was mainly in Kupffer cells; with higher doses more Fe was also detected in parenchymal cells. Two indices related to the availability of iron, IRP-IRE binding and accumulation of protoporphyrin, the substrate for ferrochelatase, were decreased at much lower Fe dextran doses than those required to produce uroporphyria. We conclude that while increasing CYP1A2 increases the sensitivity of C57BL/6 mice for uroporphyria to that of SWR mice, merely increasing hepatic Fe to the same level does not. Other strain specific factors, especially in Fe metabolism may be involved. This work was supported by the VA and NIH (ES06263, ES 06321, AG14731, DK47219).

608 CYTOCHROME P450 REDUCTASE MUTANTS WITH ALTERED COFACTOR SPECIFICITY (NADPH *VS.* NADH).

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Cytochrome P450 reductase (CPR) is responsible for electron transfer from NADPH to the cytochromes P450 and other microsomal monooxygenases. CPR has an unusually high specificity for NADPH (*vs.* NADH). These two cofactors differ only in the presence of a 2' phosphate instead of a hydroxyl group (OH) on the adenine ribose of NADPH, and thus this moiety must provide the target for cofactor discrimination. We have used site-directed mutagenesis to identify several residues that discriminate between the 2' phosphate of NADPH and the 2' hydroxyl of NADH; amino acids were chosen for mutation based on the crystal structure of ferredoxin NADP+ reductase in conjunction with sequence alignments of NADH-specific enzymes. Five single mutants (S596D, R597M, E598A, K602W, and W677A) and four double mutants (S596D-R597M, S596D-K602W, R597M-W677A, K602W-W677A) were generated. Most notably, S596D, S596D-R597M, and S596D-K602W show a greater than 23, 000-fold shift in Km value from NADPH to NADH. W677A, S596D-R597M, R597M-W677A, and K602W-W677A mutants show a higher kcat with NADH than with NADPH. The W677A mutant shows the highest overall catalytic efficiency (kcat/Km) with NADH, with a $kcat^{NADH}$ of ~3000^{-1} (comparable to the wild-type enzyme with NADPH) and a Km^{NADH} of ~100 μM, but is inhibited by NADPH concentrations as low as 2.5 μM. To determine if mutations to the 2' phosphate binding region of P450 reductase might allow activity with NADH without inhibition by NADPH, we tested K602W-W677A along with the W677A enzyme in the presence of 2.5 μM NADPH. K602W-W677A retains significant activity in the presence of 2.5 μM NADPH, a concentration that causes complete inhibition of W677A. This enzyme may be suitable for use in *in vivo* applications. This research was supported by NSF Grant MCB-9808636 and NIH Training Grant ES-07266.

609 GENDER DIFFERENCES IN THE METABOLISM OF ACRYLONITRILE TO CYANIDE IN MICE: ROLE OF CYTOCHROME P4502E1 (CYP2E1) AND EPOXIDE HYDROLASE (EH).

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Acrylonitrile (AN) is a potent toxicant and a known rodent carcinogen. AN may undergo direct conjugation with glutathione or oxidation to form cyanoethylene oxide (CEO). CEO is metabolized to yield cyanide *via* several proposed pathways including epoxide hydration. Further, male mice were reported to be more sensitive to the acute toxicity of AN than females. The objectives of this work include the study of the importance of CYP2E1 and EH in the metabolism of AN to cyanide and the role of these enzymes in the gender differences in AN-induced acute toxicity. Male and female wild-type (WT) and CYP2E1-null mice received AN at 0, 2.5, 10, 20, or 40 mg/kg by gavage. Blood and tissue cyanide concentrations were measured at 1 or 3 hr after dosing. Present results showed that cyanide levels in blood and tissues of AN-treated WT mice were significantly greater than controls and increased in a dose-dependent manner. In contrast, cyanide levels in the blood and tissues of AN-treated CYP2E1-null mice were not statistically different from those of vehicle-treated mice. Significantly higher levels of AN-derived cyanide were detected in the blood and tissues of WT male mice than in females, which may explain the higher sensitivity of male mice to AN-induced toxicity. Western blots of microsomal and cytosolic liver protein from WT male and female mice showed that while there was negligible differences in CYP2E1 expression, higher levels of EH were detected in male mice. Differences in the expression of EH may contribute to elevated metabolism of CEO to cyanide and to the greater sensitivity of male mice to AN-induced toxicity. In summary, these data demonstrated that CYP2E1 is the only enzyme responsible for the oxidative metabolism of AN, and suggest that metabolism of CEO *via* EH may play a major role in the formation of cyanide in mice. Further, gender differences in the expression of EH may explain the higher cyanide levels and the more severe toxicity observed in male *vs.* female mice.

610 DEHYDROEPIANDROSTERONE (DHEA) TARGETS MULTIPLE NUCLEAR RECEPTORS TO MODULATE CYTOCHROME P450 GENE EXPRESSION.

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DHEA is a C-19 steroid produced by the human adrenal gland. Administration of DHEA at pharmacological doses produces diverse biological effects, including chemoprotection in multiple cancer models, and amelioration of obesity, diabetes, and lupus in animal models and in humans. We have previously shown that in rats DHEA treatment modulates expression of multiple cytochrome P450 (CYP) enzymes. DHEA treatment increases expression of members of the CYP4A and CYP3A subfamilies and decreases expression of CYP2C11. In the current study, we examined the role of nuclear receptors in modulation of CYP expression by DHEA. DHEA is known to activate a member of the nuclear receptor superfamily, peroxisome proliferator activated receptor α (PPARα), to modulate expression of some genes, including CYP4A. PPARα-null mice were utilized to examine the role of this receptor in expression of CYP3A. In wild-type mice, DHEA-sulfate (150 mg/kg) induced CYP4A and CYP3A mRNA by 4- and 1.5-fold, respectively, compared to controls. In PPARα-null mice, induction of CYP4A by DHEA was lost while CYP3A induction was 1.5-fold, as in wild-type. Because CYP3A can be regulated by another nuclear receptor family member, the pregnane X receptor (PXR), we tested the ability of DHEA and its metabolites to activate PXR. A reporter gene containing two copies of the PXR responsive element from the CYP3A23 gene was transiently transfected into CV-1 cells in the presence or absence of PXR expression vector. DHEA and its metabolites androstenedione and androstenediol were activators of PXR in these assays. We also examined the involvement of nuclear receptors in DHEA's ability to suppress CYP2C11. In cell-based assays, DHEA suppresses CYP2C11 in a PPARα-independent manner. Taken together, these results indicate that DHEA can act through the nuclear receptors PPARα, PXR, and likely additional members of the nuclear receptor superfamily to modulate expression of CYPs. Supported by DK54774 (RAP) and F32 ES05927 (SLR).

611 CYTOCHROME P450 EXPRESSION DURING THE GASTRIC CARCINOGENIC PROCESS.

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Gastric Cancer (GC) is the second most common cancer worldwide. In Mexico, gastric cancer has occupied the second and third places among neoplasm death rates for many years. GC is classified according to the histological type in intestinal and diffuse (Lauren's Classification). It is known that intestinal GC is preceded of a series of histological changes that include active and inactive chronic gastritis, atrophy and intestinal metaplasia and finally gastric adenocarcinoma. Intestinal type GC is strongly related to environmental factors and the infection caused by *Helicobacter pylori* . The gastric tract is exposed to a large amount of carcinogens. It has been proved that 90% of all xenobiotics pass throughout it, most of them subject to be bioactivated by cytochrome p450 enzyme family (CYP). Our objective is to demonstrate that there is a CYP differential expression related to inflammation in the different histological progression stages leading to intestinal type GC. We sampled 244 gastric mucosal tissue specimens from the proposed gastric carcinogenesis model stages to be analyzed by immunohistochemistry performed with CYP 2E1

and 3 A4 polyclonal antibodies . *Helicobacter pylori* status, drug intake and smoking habit information were collected. The results were assessed according to the presence or absence of immunostaining using standard light microscope. Differences in staining proportions were tested with square χ test. Briefly, we found 2E1 and 3A4 CYP expression in normal tissues. This expression decreases in active and inactive chronic gastritis. A 95% of intestinal metaplasia analysed tissued exhibited 2E1 CYP expression while only 23% was found in GC specimens. CYP 2E1 and 3A4 is inversely correlated to *Helicobacter pylori* infection.

612 CYP1B mRNA EXPRESSION IN TWO RELATED CATFISH SPECIES.

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CYP1B1 is a P450 gene that in mammals is involved in the metabolism of polycyclic aromatic hydrocarbons (PAHs) and estradiol to potentially toxic intermediates. This gene was recently cloned in fish (scup and plaice). The objective of our study is to characterize CYP1B in channel catfish (*Ictaluras punctatus*, CC) and brown bullhead (*Ameriurus nebulosus*, BB). These species were selected because of their established differences in sensitivity to PAH-induced carcinogenesis; BB is the more sensitive species. An 861 nucleotide sequence to the polyA tail was cloned from CC liver that was 67% similar to the human and plaice CYP1B1 protein sequences (104 of the 183 residues were shared by all 3 species). In a 459 nt sequence spanning two exons, there was only one amino acid residue different between the two catfish species. Tissue distribution was investigated in fish exposed to control and 20 mg/kg benzo(a)pyrene (BaP). RT-PCR analysis indicated a 207 nt CYP1B band in gill, blood, gonad, kidney, and liver of BB and CC. When standardized against beta-actin, there appeared to be an induction by BaP in some CC tissues but not liver where CYP1B was lowest. Complete Dose-Response curves for CYP1B mRNA induction by BaP and TCDD exposure of primary hepatocytes and gill cells are being established with real time quantitative PCR. Using semiquantitive RT-PCR, neither TCDD nor BaP caused significant induction in BB hepatocytes, however BaP caused CYP1B some induction in CC hepatocytes. Ultimately, we will compare CYP1B induction responses in BB and CC to determine if differential CYP1B activity could have a role in species differences in PAH susceptibility.

613 ASSESSING THE ROLE OF CYP3A5 AND CYP3A7 IN DRUG-DRUG INTERACTIONS.

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CYP3A5 is present in significant quantities in 20-60% of human livers, whereas CYP3A7 is most abundant in fetal liver. The role of these enzymes in drug interactions is not well understood. A panel of 16 compounds was tested for potential to inhibit dealkylation of 7-benzyloxy-4-trifluoromethylcoumarin (BFC) catalyzed by cDNA-expressed CYP3A5, CYP3A7, CYP3A4 and human liver microsomes (HLM). CYP1A2 was used as a non-CYP3A comparator. Three of 16 compounds exhibited 50% inhibition of CYP1A2 activity (IC50) whereas at least 13 of 16 compounds exhibited 50% inhibition of CYP3A4, CYP3A5, CYP3A7 and HLM activity. α-Naphthoflavone inhibited CYP1A2, but activated all CYP3A enzymes as well as HLM catalysis. The IC50 values for all CYP3A enzymes and HLM correlated well with each other ($r > 0.77$). We show that inhibitor specificity for CYP3A5 and CYP3A7 is similar to CYP3A4 but inhibitor potency for these enzymes is on average, 45-fold less than with CYP3A4. Since HLM is a mixture that includes at least two CYP3A enzymes, expect intermediate potency when using HLM as an enzyme source in CYP3A4 inhibition assays. Indeed, this was observed. Because of similarities in inhibitor specificity between CYP3A4 and CYP3A7, CYP3A7-mediated drug-drug interactions and/or drug-endobiotic interactions in fetus may exhibit a specificity profile similar to that of CYP3A4 in adults, however, inhibition potency appears to be at least an order of magnitude less with CYP3A7.

614 DIESEL EXHAUST PARTICLE-INDUCED ALTERATIONS OF PULMONARY PHASE I AND PHASE II ENZYME SYSTEMS.

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Several environmental pollutants including polycyclic aromatic compounds induce carcinogenesis through the activation of xenobiotic metabolic pathways. The xenobiotics require bioactivation by phase I enzymes to induce carcinogenicity, while phase II enzymes contribute to resistance to chemical toxicity. The purpose of this study was to investigate the effects of diesel exhaust particle (Department) on phase I and phase II enzymes. Male rats were intratracheally instilled with saline (vehicle control) or a single dose of Department or carbon black (CB) at 5, 15, or 35 mg/kg body weight. CB was used as a control for the particulate carbon core of Department. At 1, 3, or 7 days post-exposure, lung microsomes and cytosol were prepared. The activities of CYP1A1 and CYP2B1 (Phase I enzymes) in microsomes and GST and catalase (phase II enzymes) in the cytosol were determined. Enzyme protein levels were determined by Western blot analysis. Department exposure at 5, 15, or 35 mg/kg, but not CB, significantly elevated CYP1A1 protein levels at 1 and 3 days post-exposure compared to the control. CYP1A1 activity was also increased with 15 and 35 mg/kg Department at 1 day post-exposure. On the other hand, both Department and CB exposures caused a significant decrease in CYP2B1 protein levels at 15 and 35 mg/kg with a concomitant attenuation of CYP2B1 enzyme activity. At 1 day post-exposure, both Department and CB significantly decreased the GST-Pi protein level at all doses tested with a significant attenuation in GST activity at 15 and 35 mg/kg. Catalase activity was significantly decreased by Department or CB (35 mg/kg) exposure at 1 and 7 days. These data suggest that the organic components of Department induce CYP1A1, while the carbon core attenuates CYP2B1, GST and catalase activities. The Department-induced alterations in the phase I and phase II enzyme pathways may play a significant role in pulmonary toxicity/carcinogenicity.

615 METABOLISM AND TOXICITY OF THE FUNGICIDE THIRAM AND ITS METABOLITES IN RATS.

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Thiram (tetramethylthiuram disulfide) is a dithiocarbamate compound widely used as an agricultural fungicide, antioxidant in rubber/rayon industry and antibacterial agent in medicine. The objective of this study was to examine whether thiram and its two metabolites, dimethyldithiocarbamate (DMDC) and carbon disulfide, interact with hepatic cytochrome P450 (CYP) isozymes and cause hepatic damage in rats. These compounds were individually administered i.p. to rats at two equimolar doses (0.1 and 0.5 mmol/kg) and the animals were sacrificed 3 and 24 hr after treatment. At 3 hr, there was a significant inhibition of CYP1A1 caused by only the higher dose of thiram and DMDC whereas the inhibitory effect was seen with both doses of carbon disulfide. On the other hand, CYP2E1 was inhibited by all doses of all compounds. CYP2B1 was not affected by DMDC, but was inhibited by higher dose of thiram and both doses of carbon disulfide. The activity of CYP3A2 was decreased by only the higher dose of carbon disulfide and not by others. The results of 24 hr treatment indicated that CYP 1A1 and 2E1 were inhibited only by higher dose of thiram, CYP 2B1 and 2E1 were induced by higher dose of carbon disulfide and CYP3A2 remained unaffected by all doses of all three compounds. The higher dose of thiram elevated the levels of serum SDH and ALT at 24 hr. Further assessment of liver damage was done histopathologically, and only the higher dose of thiram produced mild to moderate hepatic cell necrosis 24 hr after treatment. In summary, the results suggest that these compounds are metabolized by CYP 1A1, 2B1 and 2E1, but not by 3A2, and only thiram is hepatotoxic. Supported by NIH/MBRS S06GM08091.

616 INCREASED CYP2E1 ACTIVITY AND ENHANCED SYSTEMIC OXIDATIVE STRESS AFTER ENDOTOXIN IN HUMANS.

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Reactive oxygen species (ROS) are important toxic mediators in the pathophysiology of systemic inflammation. Previous studies have shown systemic oxidative stress in animal models of inflammation. Usually activities of hepatic P450s are decreased. However, we previously reported that CYP2E1, a P450 that leaks ROS, is not decreased in humans after LPS. To further evaluate CYP2E1 and oxidative stress in the human LPS model, human volunteers were given LPS. Methods: Six healthy, non-smoking men were given two consecutive daily injections of E. coli lipopolysaccharide (LPS) as a safe reproducible model of inflammation. In a control study each subject was monitored after saline injection. Oral and formation clearances of chlorzoxazone (CZX) as a measure of hepatic activity of CYP2E1 were assayed. Urinary F2-isoprostanes, whole blood GSH, peripheral blood leukocyte NF-κB activation, and plasma nitrate/nitrite concentration were measured as markers of systemic oxidative stress, as well as plasma TGF-*beta*1 and hyaluronic acid concentration. Results: Pharmacokinetic data of CZX clearance showed increases in the activities of CYP2E1 after LPS administration. Statistically significant two to three fold increases in urinary F2-isoprostanes were found between 0-6 hr after each dose of LPS. Plasma nitrate/nitrite concentration reached peak values of 86.0 ±36uM and 70.8 ±18uM between 3 and 6hr after first and second LPS dose respectively, while the control value was 40.1 ± 18uM. The profiles of changes in leukocyte NF-κB (p50 and p65), human plasma TGF-*beta*1 and plasma

hyaluronic acid concentrations at early time points after each LPS administration exhibited transient but significant increases that returned to normal values 24 hr after treatment. Conclusion: Our results indicate an increase in hepatic CYP2E1 activity with low-dose LPS in humans that is associated with enhanced but transient measures of systemic oxidative stress.

617 PHENYLETHYLISOTHIOCYNATE (PEITC) REDUCES 2-AMINO-1-METHYL-6-PHENYLIMIDAZO[4, 5-B]PYRIDINE (PHIP) DNA ADDUCT FORMATION BY INHIBITION OF CYTOCHROME P450 DEPENDENT METABOLISM.

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Exposure to potent heterocyclic amine mutagens, such as PhIP, result in DNA adducts that correlate with tumor formation in target tissues of rats. Consumption of cruceferous vegetables, which contain PEITC, appear to reduce the carcinogenic potential of heterocyclic amines. The mechanism by which PEITC reduces the carcinogenic potential is unknown. To determine if chemopreventitives interfere with PhIP bioactivation, microsomes from rat liver, prostate and lung were prepared to measure the metabolism of PhIP in the presence of PEITC. In a separate experiment rats were administered PEITC prior to a PhiP exposure and DNA adducts were measured by accelerator mass spectrometry. Results: Microsomes prepared from neither rat prostate, nor lung tissue generated oxidation products of PhIP. In contrast, liver microsomes generated multiple oxidation products from PhIP, including NOH-PhIP, an intermediate that results in DNA binding. PhIP metabolism in liver was inhibited by PEITC. With particular significance PEITC abolished the production of NOH-PhIP, while less reactive metabolites were still produced. When rats were administered PEITC prior to PhIP exposure, DNA adducts were reduced. We conclude: 1) NOH-PhIP is not generated by cytochrome P450 at detectable leves in extra-hepatic tissue 2) PEITC inhibits cytochrome P450 dependent metabolism of PhIP, and 3) inhibition the metabolic activation of PhIP to reactive intermediate such as NOH-PhIP, leads to the reduction of DNA Adducts. This work was performed under the auspices of the U. S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48. Research supported by USDOD USAMRDC # 45559FLD

618 USE OF REAL-TIME QUANTITATIVE REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION (QRT-PCR) METHODOLOGY TO STUDY THE INDUCTION OF CYTOCHROME P450 (CYP) mRNA LEVELS IN RAT LIVER.

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In this study QRT-PCR (TaqMan®) methodology has been employed to study the induction of CYP mRNA levels in rat liver and in cultured precision-cut liver slices. Fluorigenic (TaqMan®) probe/primer combinations specific for a range of CYP cDNAs were designed using Primer Express® software. The probe/primer sets were selected to span exon junctions in order to minimise the effect of any contamination from genomic DNA. Probes for CYP genes utilised a FAM reporter while the house-keeping (albumin) probe was labelled with VIC allowing duplex analysis for both CYP and albumin in the same reaction. To test the specificity of the TaqMan® probes and primers male Sprague-Dawley rats were treated with either a single 500 mg/kg ip dose of Aroclor 1254 (ARO) or four daily oral doses of 50 and 75 mg/kg/day dexamethasone (DEX) and methylclofenapate (MCP), respectively. Total RNA was extracted from liver samples from control rats and pooled samples from ARO, DEX and MCP treated rats and cDNA prepared. A series of duplex PCR amplifications was conducted using an ABI PRISM™ 7700 Sequence Detector. Compared to albumin (as a house-keeping gene) levels of CYP1A1, CYP1A2, CYP3A1, CYP3A2 and CYP4A1 mRNA were induced by 51, 44, 49, 13 and 62 fold, respectively. To assess CYP mRNA induction *in vitro*, rat liver slices (diameter 10 mm, thickness around 250 µm) were prepared using a Krumdieck tissue slicer. Liver slices were cultured in Williams medium E containing 0.1 µM insulin, 0.1 µM DEX, 2 mM L-glutamine, 50 µg/ml gentamicin and 2.5 µg/ml fungizone in a dynamic organ culture system at 37°C under an atmosphere of 95% O_2/5% CO_2. Treatment with ARO, but not MCP, for 24 hr markedly induced levels of CYP1A1 and CYP1A2 mRNA, whereas MCP induced levels of CYP4A1 mRNA. These results demonstrate the usefulness of TaqMan® QRT-PCR methodology for studying the induction of rat CYP mRNA levels *in vivo* and *in vitro*.

619 MEASUREMENT OF HEPATIC ENZYME INDUCTION AND β-OXIDATION IN RATS DOSED WITH CLOFIBRATE.

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The goals of this study were to evaluate the effects of various doses of clofibrate on inducible liver enzymes in the rodent and to assess possible increases in peroxisome proliferation at the higher doses. Groups of 5 male and 5 female [Crl:CD®(SD)BR (Sprague-Dawley)] rats received either 0, 300, 450 or 600 mg/kg/day clofibrate s.i.d. for 14 days. The animals were sacrificed, and the livers removed and frozen. Subsequently the livers were homogenized, and supernatants were prepared and later assayed for peroxisome proliferation based on cynanide-insensitive β-oxidation of palmitoyl CoA. In comparison with control animals, palmitoyl CoA oxidation increased significantly ($p < 0.01$) in both sexes at 300 mg/kg, but appeared to plateau at 450 and 600 mg/kg, indicating that a 300 mg/kg dose of clofibrate was adequate to generate an optimal response in the rat. Concurrently, liver microsomal samples were prepared from the same livers and later assayed for microsomal cytochrome P450, NADPH cytochrome c reductase, ethoxyresorufin O-dealkylase (EROD), methoxyresorufin O-dealkylase (MROD), pentoxyresorufin O-dealkylase (PROD), p-nitroanisole O-demethylase (P-NOD), p-nitrophenol hydroxylase (NP), and ethylmorphine N-demethylase (EMD) activities. For some of these endpoints, significant ($p < 0.05$) differences were found between the control means and those means obtained from animals dosed with clofibrate. In both sexes, significant elevations in PROD and P-NOD were seen at all three dose levels. Elevated cytochrome c reductase was noted in both sexes at the high dose. Both EROD and MROD were essentially unaffected in males and females. In males, elevations of total P450 were observed at 300 and 450 mg/kg. In females, EMD levels rose significantly at all three doses, and increases were also seen in NP at 300 and 450 mg/kg. Based upon results for several inducible rodent cytochromes, this study demonstrates that clofibrate is a hepatic microsomal enzyme inducer of CYP2B1 and/or CYP2B2 and CYP2E1 in both sexes and CYP3A1 in females.

620 IRON DEFICIENCY CAUSES MANGANESE ACCUMULATION IN DEVELOPING RAT BRAINS.

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Iron (Fe) deficiency (ID) is a prevalent nutritional disorder affecting nearly 2 billion people worldwide. It is well known that ID represents a risk factor for toxicity due to metal exposure (e.g., aluminum, cadmium, lead). Since Fe and manganese (Mn) share similar transport mechanism(s), studies were carried out to test the hypothesis that dietary ID leads to increased Mn accumulation in the brain, potentially predisposing the central nervous system (CNS) to toxicity. Previous studies have shown that ID increases brain Mn, but specific regional changes have, as of yet, not been reported. Thus, the goal of this study was to examine the effect of ID on [Mn] in Mn-rich brain regions. Twenty-one day old male Sprague-Dawley rats were fed for 6 weeks one of three semi-purified diets (AIN-93-G), (35 mg Fe/Kg, 10 mg Mn/Kg diet, CN n=7), (3 mg Fe/Kg, 10 mg Mn/Kg diet, ID n=7) and (3 mg Fe/Kg, 100 mg Mn/Kg diet, IDMn+ n=7). After 6 weeks of dietary treatment, the rats were euthanized, and the brains dissected into seven brain regions. Brain regions were analyzed for [Mn] with neutron activation analysis (NAA), and amino acid (glutamate, aspartate, glutamine, glycine, taurine, GABA) concentrations were measured with HPLC. Both Fe-deficient diets caused significant ($p<0.05$) increases in [Mn] in six of the brain regions compared to controls (ranging from cerebellum, 31%-globus pallidus 60% increase). The hippocampus was the only brain region in which the IDMn+ group accumulated significantly more Mn than both the CN and ID groups (CN<ID<IDMn+). While multiple alterations in brain regional amino acid concentrations were observed, two significantly correlated with CNS [Mn]. In the substantia nigra, [Mn] significantly correlated with increased taurine concentration ($r=0.65$; $p<0.05$), whereas in caudate putamen [Mn] negatively correlated with decreased g-amino butyric acid (GABA) ($r=-0.54$; $p<0.05$). These data show that ID increases brain Mn in a heterogeneous fashion, and that ID is a significant risk factor for CNS Mn accumulation. (Supported by NIEHS 10563).

621 PRENATAL LEAD (PB) EXPOSURE AND SCHIZOPHRENIA: METHODOLOGY FOR EXAMINING THE POTENTIAL RELATIONSHIP.

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Schizophrenia is a mental disorder affecting approximately 2.8 million Americans, characterized by hallucinations, delusions, emotional and social withdrawal, and disorganized thinking. The causes of the disease are thought to include hitherto unspecified genetic and environmental risk factors. Prior studies suggest that disruptions in prenatal development may be risk factors. The current study was designed

to test the hypothesis that exposure to a specific neurodevelopmental disruptor (Pb) during the 2nd trimester of pregnancy might increase the risk of developing the disease. Serum samples from a cohort of 19, 044 pregnancies were collected between 1959-1966. Samples of serum were taken at each trimester and at delivery, respectively. Samples were stored at 20 C until the present day for an average storage time of 38 years. Diagnosis of schizophrenia spectrum disorder in the children born to this cohort yielded a total of 71 cases. A method to quantitate a biological marker of Pb exposure, i.e. delta-aminolevulinic acid (d-ALA) has been developed. Serum samples were processed and treated with acetylacetone and derivatized with formaldehyde to produce a fluorescent molecule. The samples were fractionated on a reverse phase C18 column and assayed by a fluorescence detector with an excitation wavelength set at 360 nm and an emission wavelength of 470 nm. Our method improves the detection limit by a factor of 10, ultimately yielding 2.4 ng/mL of d-ALA. d-ALA values greater than (and less than) 10 ng/ml agree well with BPb values greater than (and less than) 14 ug/dL. This work is currently underway on the primary study.

622 COGNITIVE EFFECTS IN LEAD EXPOSED CHILDREN FROM URBAN AREAS IN THE REGION LAGUNERA, MEXICO.

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There is known that exposure to low doses of lead can cause cognitive effects in children. This population study was based in a previous cross sectional survey, where it was demonstrated that heavy metal contamination in soil, dust, and air around a smelter site by far exceeded background levels, and that a high proportion (> 90 %) of children living in the neighborhood had lead in blood concentrations (PbB) above 10 µg/dL (J. Toxicol. Environment Health. Part A, 62:417-429, 2001). From a initial number of 394 scholar children, 285 were recruited and their parents filled a consent letter form, to be studied by psychometric tests, particularly the Wechsler Intelligence Scale for Children - Revised (WISC-R) for Mexican children in Spanish. Diet, socioeconomic status, hygienic habits, and other variables were assessed by questionnaire. Gender distribution was 151 male, and 134 female, aged 6 – 14 years. The mean (range) of PbB was 19.13 µg/dL (3.9 – 58.5 µg/dL). Same parameters for psychometric index were: IQ: 100.32 (75.0 – 134.0) and for equivalent mental age: 8.15 years (5.0 – 11.5 years). Non parametric Spearman's correlation were significant between lead and IQ (ρ = - 0.169; p = 0.004) and for lead and equivalent mental age (ρ = -0.206; p< 0.0001). Socioeconomic status and education levels were co-variables for PbB and IQ, however, when the results were adjusted by the co-variables, the relationships among PbB and IQ, and equivalent mental age remain significant. The results confirm that chronic lead exposure have cognitive effects on children, particularly in mental maturity. These results will be useful for follow-up studies and set up epidemiological surveillance programs in this population.

623 HEME BIOSYNTHESIS IMPAIRMENT IN CHILDREN CHRONICALLY EXPOSED TO LOW DOSES OF LEAD IN A SMELTER COMMUNITY IN MEXICO.

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Metal contamination in soil, dust and air around a smelter site has resulted in increased lead body burden in children living in the vicinity of the major smelter complex in Latin America, located in Torreón, Coahuila, northern Mexico. Children with lead in blood concentrations (PbB) over 10 *micro*|g/dL (value considered as indicative of lead poisoning) are at risk of suffering adverse effects of lead. Heme biosynthesis can be disturbed by lead exposure. Several heme biosynthesis enzymes or intermediate metabolites has been used as biomarkers in lead toxicity. The aim of this work was to assess two of the main lead toxicity biomarkers related with heme synthesis, particularly we evaluate the activity of the 5- aminolevulinic dehydratase (ALA-D; assayed by HPLC) and zinc protoporphyrin (ZPP; measured by fluorescence) in 270 children aged from 6-7 years old. Children were recruited from nine primary schools, 2 km around the smelter. Diet, socioeconomic status, hygienic habits, and other variables were assessed by questionnaire. Median (range) of PbB in children was 11.18 *micro*|g/dL (3.0 – 43.82 mg/dL), whereas for ZPP was 63.0 mmol of ZPP per mol of Hb (27.0 – 452.0 mmol of ZPP per mol of Hb), and for ALA-D was 147.0 nmol PBG / mL RBC x h (28.9 – 332.9 nmol PBG / mL RBC x h). There was a significant inverse relationship [r = -0.467; p< 0.00001; equation: Log (y) = 0.869 (log x) + 3.167] between PbB and ALA-D, and a significant positive relationship between PbB and ZPP [r = 0.443; p< 0.00001; equation: $y = 2.05 (x) + 42.22$]. These relations remain significant even adjusted by hemoglobin. These results showed that heme biosynthesis biomarkers, particularly ALA-D activity and ZPP in blood are useful biomarkers of damage for lead toxicity in children exposed to low doses of the metal.

624 LOW-LEVEL POSTWEANING LEAD (Pb) EXPOSURE: ALTERATIONS IN TIMING BEHAVIOR.

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Several clinical and experimental studies have suggested that low-level lead (Pb) exposure produces impulsive behavior and/or delay aversion. In addition, Pb exposure is associated with deficits in temporal behaviors, i.e. fixed-interval schedules. However, no studies have been conducted to determine if these Pb effects are related to differences in timing mechanisms. To test this hypothesis, rats exposed chronically from weaning to 0, 50, or 250 ppm Pb acetate in drinking water were tested on a temporal bisection task. Rats were trained to respond on one lever after the presentation of a 4-s ("short") tone and on another lever after the presentation of a 16-s ("long") tone. Once all subjects reached a criterion of 80% correct for both the 4 and 16-s tones, intermediate tones (5.2, 6.4, 8.0, 10.0, and 12.8 s) were added to the trials procedure. For each rat, the percentage of "long" responses at each signal duration was calculated, and psychophysical functions were determined by using least squares. The point of indifference, defined as the stimulus duration at which a rat responds "long" 50% of the time and the difference limen, an estimate of the threshold to detect differences between two time lengths were calculated from the individual psychophysical functions. Pb exposure produced a decrease in the PI without altering the DL. Pb exposure had no effect on the ability to discriminate the 4 and 16-sec tones. These results indicate that Pb produced an increase in clock speed without altering threshold sensitivity or discrimination ability and support the hypothesis that alterations in timing mechanisms play a role in some Pb-related behavioral deficits. Funded by NIEHS (ES010992).

625 EFFECTS OF CHRONIC LEAD EXPOSURE ON HIPPOCAMPAL BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) CONCENTRATIONS IN RATS.

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Previous studies have established that chronic developmental lead (Pb) exposure impairs the magnitude and duration of long-term potentiation (LTP) and the amount of stimulus-evoked hippocampal glutamate release. Other evidence indicates that induction of LTP is dependent on appropriate BDNF expression and release, and BDNF also potentiates glutamatergic transmission by increasing the efficacy of glutamate release. Moreover, chronic Pb exposure has been reported to decrease neurotrophic factor gene expression (Schneider et al., 2001). This study examined the production of hippocampal BDNF protein in rats chronically exposed from birth. Pregnant Long-Evans dams were exposed to 0.2% Pb in drinking water beginning at gestational day 16 and exposure was continued throughout lactation with male offspring continued on the same solution until testing. Previous studies have shown that this exposure protocol produces blood and brain Pb values of 40 ug/dl and 378 ng/g tissue, respectively (Gilbert et al., 1999). At 8 months of age rats were sacrificed and dorsal hippocampi and parietal cortices were dissected and sonicated. Cell lysates were clarified by centrifugation, briefly acidified in phosphate-buffered saline, and BDNF quantified by a microplate-based sandwich enzyme immunoassay; signal was determined by the absorbance at 450 nm. Total protein was determined by the bicinchoninic acid method. Hippocampal expression of BDNF protein did not differ between Pb-exposed and control animals. While alterations in BDNF activity could conceivably be an important factor in the impaired LTP and diminished glutamate release observed in tetanically-stimulated or depolarized hippocampal tissue, these data suggest that such exposure-induced changes cannot be traced to alterations in the expression of BDNF protein in unperturbed animals. (Supported by the Children's Miracle Network)

626 TOXICOLOGICAL ASPECTS OF LEAD: COGNITIVE EFFECTS IN CHILDREN AND NEUROCHEMISTRY IN YOUNG ALBINO RATS.

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Although little is known about the mechanism of lead neurotoxicity, the hazardous effects of lead exposure on the young has long been recognized. The current study investigated the effects of lead exposure on the intelligence and cognitive abilities of

children, as well as on gamma amino butyric acid (GABA) levels in the brains of young albino rats. Three hundred children of both sexes selected from different residential areas, were investigated for their blood lead level (BLL), IQ and minimental state. The clinical study on the children revealed that there was a strong relationship between the place of residence and lead exposure. Children living in rural areas had the lower blood lead levels, while those living in the urban areas had the higher blood levels. Children living in the rural areas had higher IQs and cognitive abilities, compared to those in urban areas. Fifty four young albino rats divided into 3 equal groups were exposed to 0, 100 and 1000 ppm lead acetate in drinking water respectively for 4 weeks. BLL and GABA levels were determined at the end of 2nd, 4th, and 6th weeks. No behavioral changes were noticed upon exposure to the examined doses of lead. However, lead exposure at 100 and 1000 ppm was found to elevate GABA levels. At the end of the follow up period (the end of the 6th week), GABA levels decreased toward normal control levels at the end of the 6th week although BLL remained high.

627 DEVELOPMENTAL LEAD EXPOSURE AFFECTS NEUROCHEMICAL AND IMMUNOHISTOCHEMICAL MARKERS OF THE RAT DOPAMINERGIC AND SEROTONERGIC SYSTEMS.

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Recently, we have demonstrated that exposure to lead (Pb) during pregnancy and lactation induced behavioral alterations in rats on PN23 and on PN70. Considering the role that the dopaminergic and serotonergic systems play in behaviors, this study was undertaken to investigate the effects of Pb exposure on these neurotransmiter systems. Wistar rat dams received 500 ppm of Pb acetate or 660 ppm Na acetate in the drinking water during pregnancy and lactation and the pups were euthanized on PN23 or 70. Dopamine (DA) and its metabolites DOPAC and HVA, as well as serotonin (5-HT) and its metabolite 5-HIAA were determined (ng/g of tissue) in the hypothalamus, hippocampus and striatum. Coronal sections immunostained for tyrosine hydroxylase (TH) or 5-HT were used for morphometric analyses (# of cells and volume) of the ventral tegmental area (VTA), substantia nigra (SN) and dorsal raphe nucleus (DRN). Data were analyzed by three-way ANOVA (Treatment x Age x Region). Data are presented as mean±SE. Means followed by different letters are statistically different. Theese results show that low-level Pb exposure during pregnancy and lactation induce neurochemical and morphological alterations in a brain region-specific manner that may be related to the behavioral alterations previously observed.

	PN23 Na	PN23 Pb	PN70 Na	PN70 Pb
HYPOTHALAMUS				
5-HT	627±53^{A}	910±71^{B}	709±49^{A}	677±56^{A}
5-HIAA/5-HT	0.8±0.04^{A}	0.6±0.04^{B}	0.7±0.02^{A}	0.6±0.05^{B}
HIPOCAMPUS				
DA	nd	nd	108±8^{A}	25±1^{B}
DOPAC	96±6^{A}	88±8^{B}	76±6^{C}	49±5^{D}
DOPAC/DA	-	-	0.7±0.04^{A}	0.27±0.14^{A}
5-HIAA/5-HT	1.7±0.2^{A}	1.4±0.05^{B}	1.4±0.1^{C}	1.2±0.1^{C}
STRIATUM				
HVA	1134±96^{A}	792±37^{A}	611±37^{A}	589±31^{C}
HVA/DA	0.24±0.02^{A}	0.17±0.01^{B}	0.1±0.01^{C}	0.1±0.01^{C}
Number of cells VTA	33244±1713^{A}	42272±2523^{B}	46533±2325^{C}	39403±1660^{D}
Volume SN (mm³)	4.2±0.1^{A}	4.6±0.1^{B}	6.3±0.1^{C}	6.1±0.1^{C}

628 PERINATAL LEAD EXPOSURE ALTERS INOSITOL POLYPHOSPHATE RECEPTOR BINDING IN DEVELOPING RAT BRAIN.

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Lead (Pb), an environmental neurotoxicant is known to mimic or inhibit the actions of calcium in biological systems and alter calcium mediated cellular processes. The phosphoinositide (PI) cycle is a major second messenger system that is involved in the release and regulation of calcium.Our recent studies demonstrated that the developmental Pb-exposure decreased calcium/calmodulin dependent nitric oxide synthase (NOS) activity in rat brain. In the present study, we examined the effects of Pb and NOS-modulators, L-nitroarginine (NA), 7-nitroimidazole (NI) and sodium nitroprusside (SNP) on inositol 1, 4, 5 trisphosphate (InsP3) and inositol 1, 3, 4, 5-tetrakisphosphate (InsP4) binding to membrane receptors in the cerebellum and hippocampus of Sprague-Dawley rat brain. Pb-exposure was initiated on gestation day 6 (GD6) and continued through postnatal day 21 (PND 21) for *in vivo* studies; whereas adult brains were used for *in vitro* studies. The results showed a progressive increase in the binding of InsP3 and InsP4 in both hippocampus and cerebellum during postnatal development following Pb-exposure. The *in vitro* studies also showed similar increase in the binding by Pb or NOS inhibitors (NA and NI) in a concentration-dependent manner; however, SNP (NO-donor), significantly decreased InsP3 and InsP4 binding. These results suggest that Pb alters the PI pathway leading to alterations in intracellular calcium homeostasis and provide evidence for the interrelationship between NOS and PI cycle. (Supported by NIH/GM 60853).

629 ENVIRONMENTAL ENRICHMENT REVERSES COGNITIVE DEFICITS INDUCED BY LEAD.

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Children exposed to lead (Pb2+) exhibit long-term deficits in cognitive function. A recent study has shown that chelation therapy, the primary means of treating Pb2+-poisoned children, has no significant impact on Pb2+-induced cognitive deficits (Rogan et al., N.Eng.J.Med.. 344: 1421, 2001). Thus, finding alternative intervention strategies is imperative. We have previously described that rats exposed to Pb2+ during gestation and lactation until weaning, exhibit deficits in spatial learning as young adults (Kuhlmann et al., Neurosci.Lett. 233: 101, 1997). Using this Pb2+ exposure protocol, we set out to determine if environmental enrichment (EE) affected the learning deficits induced by Pb2+. One male rat from control or Pb2+-exposed litters was randomly assigned to standard or EE housing. The latter contained platforms, tunnels, toys, a hammock and a running wheel. Standard housing consisted of one rat per cage, while EE housing had 8 rats of the same treatment per cage. Rats were reared under these housing conditions from postnatal day 21 (weaning) until the end of the water-maze spatial learning task that started at postnatal day 50. Lead-exposed rats reared in EE cages acquired the spatial learning task to the same degree as control EE rats. On the other hand, Pb2+-exposed rats reared in standard housing conditions were markedly impaired in the acquisition of the spatial learning task. Probe test data confirmed that Pb2+-exposed rats housed in an enriched environment were able to learn the task while those in standard housing did not. No group differences were noted for body weight, swimming speed or cue-test (non-spatial). These findings demonstrate for the first time that EE initiated after developmental exposure to Pb2+ is able to totally reverse the spatial learning deficits induced by Pb2+. In summary, our studies suggest a new direction for the treatment of children with learning disabilities resulting from environmental Pb2+ exposure. [Supported by grant # ES06189 to TRG].

630 ALTERED NEUROGENESIS IN THE DENTATE GYRUS OF LEAD-EXPOSED RATS: PRELIMINARY FINDINGS.

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Chronic exposure to lead (Pb2+) during development diminishes spatial learning in young adult rats [Nihei et al., Neuroscience 99: 233, 2000]. Recent studies suggest that modulation of neurogenesis in the dentate gyrus (DG) can influence the ability to learn a spatial learning task. To investigate whether Pb2+-induced spatial learning deficits are associated with altered neurogenesis in the DG, we examined cell proliferation in rats chronically exposed to Pb2+ using the thymidine analog bromodeoxyuridine (BrdU). Rats were injected with BrdU (200 mg/kg or 100 mg/kg daily) and perfused transcardially (two hours or five days, respectively) at postnatal day 50. BrdU labeled cells were counted in the suprapyramidal blade (SPB), infrapyramidal blade, dentate crest, and hilus. BrdU-labeled cells localized in the subgranular zone (SGZ) of the DG were irregular in shape and frequently arranged in clusters. Within the granular cell layer, labeled cells were seen occasionally and only after five days of BrdU injection. In rats perfused two hours after BrdU injection, cell counts showed no significant differences in any DG region as a function of treatment. On the other hand, significant treatment effects on the number of BrdU labeled cells were found in animals injected for five days. Pb2+-exposed rats exhibited a lower number of BrdU labeled cells in the SGZ of the suprapyramidal blade (24.3%, $p < 0.05$) and in the SGZ and granule cell layer of the suprapyramidal blade combined (21.8%, $p < 0.05$). Total number of BrdU labeled cells in the entire SGZ were also decreased, but the difference did not reach statistical significance ($p = 0.091$).These observations suggest that chronic developmental exposure to Pb2+ decreases net neurogenesis, possibly by reducing the survival of proliferating cells in specific areas of the DG. This alteration in DG neurogenesis may reflect a novel mechanism by which developmental exposure to Pb2+ causes deficits in spatial learning [Supported by grant # ES06189 to TRG].

631 GESTATIONAL EXPOSURE TO METHYLMERCURY RETARDS OPERANT BEHAVIOR IN TRANSITION IN ADULT RATS.

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Evidence is growing that developmental exposure to methylmercury (MeHg) has behavioral effects that extend into adulthood and aging. To study MeHg's prolonged effects on behavioral plasticity, rats were exposed to methylmercury during gestation *via* maternal drinking water containing 0, 0.5 or 6.4 ppm Hg as MeHg. Exposure began at least four weeks before mating, and continued to post-natal day 16. Brain Hg concentrations were 0.49 and 9.8 ppm at birth in littermates of the rats described here; lactation resulted in no mercury exposure. The behavior of one- and two-year old offspring was maintained under various Concurrent Variable Interval reinforcement schedules in which pressing each of two levers was maintained by independent Variable Interval t schedules of reinforcement, and t is the average inter-reinforcer interval. During the first 30 minutes of 150 min. sessions, the reinforcement rates on both levers were equal, and the ratio of left to right (L:R) lever presses approximated 1.0. After 30 min. the left lever became rich relative to the right, the right became relatively rich, or there was no change. During sessions in which one lever became rich, responding was tracked as it transitioned to reflect the new allocation of reinforcers. Terminal reinforcer ratios (L:R) used were 9:1, 4:1, 3:1, 1:1, 1:3, 1:4, and 1:9). In controls and most exposed rats, response ratios reflected reinforcer ratios by the end of the session. The transitions of many exposed rats were retarded relative to controls, and in some transitions they went in the wrong direction. The tactic used here greatly reduced the time required to study behavior in transition from a month in previous reports to a single session here. (Supported by ES06466 from NIEHS).

632 GESTATIONAL EXPOSURE TO CHLORPYRIFOS: QUALITATIVE AND QUANTITATIVE NEUROPATHOLOGICAL CHANGES IN THE FETAL NEOCORTEX.

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This study investigated the qualitative and quantitative neuropathological changes that occur in the fetal brain following gestational exposure to chlorpyrifos [(O, O'diethyl O-3, 5, 6-trichloro-2-pyridyl) phosphorothionate], a commonly used organophosphorus insecticide. Two cohorts of pregnant rats were orally dosed daily with chlorpyrifos in corn oil on gestational days 14-18. Cohort A consisted of a time course study of fetuses collected at 2, 5, 10, and 24 hours after the last dose of chlorpyrifos (0 or 7 mg/kg/day). Cohort B is a replication of Cohort A and includes a Dose-Response component at 5 hours (0, 1, 3, 5, and 7 mg/kg/day of chlorpyrifos). Fetal brain total cholinesterase, acetylcholinesterase, or butyrylcholinesterase activities were 70, 75, or 53 % of control activity 5 hours after the last 7 mg/kg dose of chlorpyrifos. Fetal brain sections stained with cresyl violet for neuropathological assessments showed overt pathology in the neocortex, consisting of vascular alterations, disorganized migratory waves, ectopic cells, neuropathological holes, and qualitative thinning of the ventricular zone and cortical plate. Neuropathological changes were evident at dosages as low as 1 mg/kg/day. Stereology used to quantify the volume of the ventricular zone, subventricular zone, and cortical plate in the fetal neocortex showed limited treatment-related changes in the volume of the subventricular zone at 7 mg/kg/day but no changes in other neocortical laminae. We conclude that repeated, gestational exposure to chlorpyrifos not only inhibits cholinesterase activity in the fetal brain, but also causes neuropathological changes in the fetal neocortex. This abstract does not reflect EPA policy.

633 PRENATAL EXPOSURE TO CHLORPYRIFOS ALTERS NEUROTROPHIN IMMUNOREACTIVITY AND APOPTOSIS IN RAT BRAIN.

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The effects of the organophosphate pesticide chlorpyrifos (CPF) on the regional distribution of three neurotrophic factors that regulate brain development and on levels of apoptosis. Pregnant Long-Evans rats were dosed daily (*p.o.*) with CPF (0, 3, 5, 7, and 10 mg/kg) in corn oil on gestational days 14-18. Fetuses were examined at 2, 5, 10, and 24 hr after the last dose. Immunohistochemistry (IHC)showed very little nerve growth factor (NGF) immunoreactivity (IR) in the fetal brain. There was significant brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) IR in the ventricular and subventricular zones fetal brains. BDNF IR was widely and spottily distributed throughout the intermediate zone and cortical plate of the control neocortex. There was a decrease in BDNF and NT-3 IR in the brains of CPF treated fetuses. These CPF-related changes in NTF-IR are consistent with quantitative changes in whole brain BDNF and NT-3 levels determined by enzyme-linked immunoabsobent assays(ELISA)'s. The IHC demonstrates that these changes are limited primarily to proliferative zones of the forebrain. Results from cell death ELISA showed the highest level of DNA fragmentation at the 7 mg/kg/day dosage at 5 hr after the last dose. Terminal transferase dUTP nick end-labeling (TUNEL) assays of fetal brains showed increased numbers of apoptotic cells in the striatum, hippocampus and cortical plate. This apparent increase in apoptosis was localized primarily to proliferative zones in the striatum and the hippocampus. The altered pattern of apoptosis in the fetal brains of CPF-exposed rats were reciprocal to changes in BDNF and NT-3 IR in proliferative and post migratory zones. This observed increase in apoptosis could be due to the changes in neurotrophin levels. Further, these changes in regional levels of neurotrophins and apoptosis with CPF exposure suggest possible alterations in cell number and function that could persist in the adult nervous system.This abstract does not reflect EPA policy.

634 CHLORPYRIFOS ALTERS GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IN THE DEVELOPING RAT BRAIN: DISRUPTION OF ASTROCYTE DEVELOPMENT?

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Despite limitations recently imposed on the organophosphorus pesticide, chlorpyrifos (CPF), concerns remain regarding its potential neurotoxicological properties in the developing nervous system. CPF inhibits brain cell replication and perturbs differentiation but the specific types of cells targeted by CPF have not yet been identified. CPF affects gliotypic cells in culture. We therefore administered CPF to developing rats and examined expression of the astrocytic protein, GFAP, using immunoblotting. Prenatal treatment increased GFAP levels in fetal brain only at CPF exposures that elicited maternal toxicity (reduced weight gain), and no persistent changes were seen as late as postnatal day (PN) 30. CPF treatment on PN1-4, at doses below the threshold for systemic toxicity (1 mg/kg/day) elicited initial suppression and rebound elevation of GFAP, limited to the cerebellum of males. Bergmann glia, responsible for guiding neuronal migration, are emerging in the cerebellum at that time, and our results may thus explain the targeting of motor function in males with this treatment regimen. When we gave CPF later, during the peak of gliogenesis (PN11-14, 5 mg/kg/day), GFAP showed initial deficits across all brain regions and in both sexes; rebound elevations (PN30) were seen in all regions in males, with the largest effect in striatum (significant for both males and females). Our results indicate that CPF targets glial development *in vivo*, so that the postnatal period is a critical period of susceptibility. The region-, sex-, and time-dependent effects on astrocytes are likely to contribute to comparably selective alterations in synaptogenesis, axonogenesis, and ultimately, behavior. Glial targeting suggests that the vulnerable period for CPF neurotoxicity extends relatively late in brain development, into the phases of synaptic plasticity, myelination, and architectural modeling. (Supported by USPHS ES10356, ES10387, and an EPA STAR fellowship)

635 DEVELOPMENTAL NEUROTOXICITY OF CHLORPYRIFOS: DEFINING THE VULNERABLE PERIOD.

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There is concern over the consequences of fetal and childhood exposure to chlorpyrifos (CPF) and related pesticides. In previous work, we found that neonatal CPF produced cell damage/loss in the brain, with abnormalities of neurotransmission and attendant behavioral anomalies. We used the same biomarkers to examine prenatal treatment "windows" to define the critical periods in which neural and synaptic development show immediate effects of CPF. One group of pregnant rats received 0, 1, 2 or 5 mg/kg daily by sc injection from gestational days (G) 9-12, during neural tube formation. A second group received 0, 1, 2, 5, 10, 20, or 40 mg/kg daily on G17-20, a peak period of neurogenesis. In the G9-12 treatment group, the threshold for maternal toxicity, defined by reduced weight gain, was 5 mg/kg; however, even this was only a transient reduction. There was no reduction in the number of fetuses assessed on G17 or G21, nor were there significant changes in fetal weight, brain weight, heart weight or liver weight. When the treatment window was shifted to G17-20, the threshold for impairing maternal weight gain was slightly higher (10 mg/kg), and although a similar threshold was seen for impairment of fetal body and liver weights, both the heart and brain were spared. DNA content, a marker for cell number, was unaffected in brain regions, although reductions were seen in the fetal liver at doses exceeding the threshold for maternal toxicity. These results stand in contrast to the robust effects seen previously for im-

mediate effects of postnatal CPF treatment on brain development, where apparently subtoxic doses nevertheless elicit reductions in brain cell number. It remains possible that defects from prenatal CPF exposure may emerge later in development; we have preliminary evidence that behavioral anomalies emerge in adolescence or young adulthood. However, in terms of immediate biomarkers of neuronal damage, it appears that later stages of development are more sensitive. (Supported by USPHS ES10387 and ES10356, and by the Leon Golberg Toxicology Fellowship)

636 NEUROCHEMICAL EFFECTS OF REPEATED DEVELOPMENTAL EXPOSURES TO CHLORPYRIFOS ON THE CHOLINERGIC NERVOUS SYSTEM.

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Neurochemical components of the cholinergic system play critical roles in the development of the nervous system. The widely used organophosphorus insecticide chlorpyrifos (CPS) has been shown to affect the levels or activities of many of these components. The effects of repeated exposures to CPS during the gestational and postnatal periods on the activities of brain cholinesterase (ChE) and choline acetyltransferase (ChAT) were studied. Pregnant Sprague-Dawley rats were orally administered CPS daily from gestation days 6-20 at doses of 0 (control; C) or 5 mg/kg/day (5). Following parturition, designated as postnatal day (PND) 0, pups were orally gavaged with CPS from PND1-21 at doses of 0 (control; CC and 5C), 0.75 (low dose; CL and 5L), or incremental doses of 0.75 to 3 mg/kg/day (high dose; CH and 5H). Pups were sampled on PND1, 6, 12, 22, and 30. ChE and ChAT activities increased about 3.3 and 11-fold from PND6 to PND30, respectively. ChE was inhibited by about 26% on PND1 in the 5C group and had returned to control values by PND6. ChE was inhibited in a dose-related manner in all treatment groups, with inhibition of about 22 and 24% on PND6 in the CL and 5L groups, respectively; and on PND12 by about 23, 51, 31, and 47% in the CL, CH, 5L, and 5H groups, respectively. ChE inhibition on PND22 was about 12, 52, 16, and 52% in the CL, CH, 5L, and 5H, respectively, and persisted in the CH (22%) and 5H (24%) groups through PND30. ChAT activity was decreased by about 11% only in the 5H group on PND22 and 30. These data suggest that exposure to CPS during the gestational and postnatal periods results in relatively persistent inhibition of ChE. In addition, exposure to CPS during the gestational period may have resulted in an increased susceptibility of these animals to decreases in ChAT activity elicited by subsequent postnatal exposures to low levels of CPS. (Partially supported by NSF EPS-9874669)

637 BEHAVIORAL EFFECTS IN TWO GENERATIONS OF RATS FOLLOWING DAILY PRE/POSTNATAL EXPOSURE TO TECHNICAL CHLORDANE.

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Male and Female Sprague-Dawley rats were used in a two-generation study to evaluate neurobehavioral toxicities of technical grade chlordane, a mammalian toxicant and formerly used pesticide, now persistent in the environment. Adult and juvenile rats (F0, F1, F2) were exposed (in replicate-1) to continuous levels of chlordane (0.0, 0.125, 0.25 and 1.0 mg/kg) by daily oral gavage. Offspring (F1 and F2) were exposed *via* dam in-utero and *via* lactation until weaning. In the F1 generation, chlordane at 1 mg/kg body weight (bw) delayed ($p < 0.05$) male and female negative geotaxis (PND 7-10), and olfactory discrimination (PND 11), but advanced both surface righting reflex (PND 3-4) and spatial learning abilities in Morris maze (PND 77); at 0.25 mg/kg chlordane advanced female Morris maze spatial learning. For F2 generation, chlordane at 1 mg/kg/bw. delayed female olfactory discrimination and fore-limb grip strength; at 0.25 mg/kg chlordane delayed male and female surface righting reflex and male hind-limb grip strength. In general, chlordane manifested greater neurobehavioral changes in the F1 generation compared to F2. The female rats were more sensitive than the males to chlordane effects (Supported by MHPF/ATSDR # U50/ATU3922660008)

638 REPRODUCIBILITY OF DEVELOPMENTAL NEUROTOXICITY PRODUCED BY PYRETHROIDS AND DDT IN NEONATAL MICE.

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Eriksson et al.(1) found that mice showed persisting changes in neurochemistry and behaviour after administration of very low doses of pesticides such as pyrethroids or DDT to the neonate. We examined the reproducibility of these effects. Three trials of a single dose of DDT (1mg/kg) to 10 day old mouse pups produced small but significant decreases ($p<0.01$ or <0.001) in neocortical muscarinic receptor density at 4 months in each trial. This effect was also seen at 0.5, 5 & 100 mg/kg, and resembled that reported by Eriksson et al. (1). After daily administration of pyrethroids (bioallethrin or deltamethrin) (0.7 or 3.5mg/kg) from the 10th to the 16th postnatal day, neocortical muscarinic receptor binding at 4 months showed no consistent changes. Behavioural tests at 4 months showed significantly delayed habituation of open field behaviour in only one trial and at one DDT dose level. Neither pyrethroid produced a change in habituation. Although our baseline values for muscarinic receptor density (813-1321 fmol/mg protein) were similar to those reported by Eriksson et al. (1), we could not reproduce the rapid habituation shown by their control mice, despite the use of the same strain and similar test conditions. We can confirm the reported developmental effects of DDT on muscarinic receptors but found that those of pyrethroids were not reproducible. Reported behavioural effects were also not reproducible, although the latter may be due to baseline differences. We conclude that although neonatal exposure to DDT produces reproducible and lasting changes in neurochemistry, pyrethroids do not produce a robust effect, possibly due to their rapid disposal from the body or to a dependence on as yet undefined variables. (1) Eriksson P, Ahlbom J & Fredriksson A (1992 Brain Res. 582, 277-281

639 EFFECTS OF DEVELOPMENTAL EXPOSURE TO HEPTACHLOR ON THE CHOLINERGIC SYSTEM IN RATS.

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Heptachlor is an environmentally persistent cyclodiene pesticide which is a known antagonist of the γ-aminobutyric acid $(GABA)_A$ receptor. Since GABA is a trophic factor for the development of other neurotransmitter systems (Lauder *et al.*, Perspectives in Developmental Neurobiology 5:247-259, 1998) exposure to heptachlor may affect neuronal development. To determine the developmental effects of heptachlor, one group of rats was orally dosed with 0 or 4.2 mg/kg/day of heptachlor from gestational day (GD) 10 to postnatal day (PND)21, and another group was dosed with 0, 0.3, or 3 mg/kg/day from GD10 to PND42. From PND2-5 there was a delay in righting reflex ontogeny in pups exposed gestationally to 3 mg/kg/day of heptachlor. In adult rats dosed with 3 mg/kg/day of heptachlor from GD10 to PND42, there was a significant decrease in muscarinic acetylcholine receptor binding in the cerebellum, but not in the striatum or frontal cortex. In adult rats dosed with 4.2 mg/kg/day of heptachlor from GD10 to PND21, there was a decreased hypothermic response to oxotremorine. An increased response to scopolamine on motor activity was observed in adults exposed to 3 mg/kg/day from GD10 to PND42. These data suggest a functional consequence of fewer muscarinic acetylcholine receptors. Thus, exposure to heptachlor during development resulted in a developmental delay and long-lasting alterations of the cholinergic system. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

640 EFFECTS OF CHRONIC BLOCKADE OF SODIUM CHANNELS AND NMDA RECEPTORS DURING DEVELOPMENT ON THE ACQUISITION AND PERFORMANCE OF SEVERAL COGNITIVE FUNCTION TASKS IN RATS.

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The effects of MK-801 (a relatively selective NMDA receptor antagonist), phenytoin (a sodium channel blocker) and a combination of the two on the acquisition and performance of several complex, food-reinforced operant behaviors were assessed in juvenile female rats. Audio/visual discrimination (AVD), learning (LRN), and motivation (MOT) were modeled using conditioned position responding, incremental repeated acquisition and progressive ratio tasks, respectively. LO and HI doses of both drugs (0.1 and 1.0 for MK-801, 30 and 150 mg/kg/day for phenytoin, PHT) and LO and HI dose combinations (COMB) of both drugs were administered once daily, 7 days/week by oral gavage beginning on postnatal day (PND) 23. M-F behavioral assessments began on PND 27 and are ongoing (>PND 122). Over the duration of the study, HI MK-801 and HI COMB subjects exhibited decreased body weight gains and performance in all three behavioral tasks (the performance and body weight gain of all other groups was similar to that of controls). For these two affected groups, response rates were decreased in all tasks, indicating decreased motivation and/or motoric capabilities. Accuracy was decreased for both these groups in the AVD task, but only for the HI MK-801 group in the

LRN task. Thus, chronic treatment during development with LO or HI PHT, LO MK-801 or LO COMB had little or no effects, while HI MK-801 and HI COMB differentially affected accuracy of performance of two cognitive function tasks. These data suggest that, during development in the rat: blockade of sodium channels is well tolerated and that blockade of NMDA receptors alone has significant adverse consequences for the development of important cognitive functions.

641 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) IMPAIRS SPATIAL LEARNING AND ALTERS HIPPOCAMPAL MORPHOLOGY IN FEMALE MICE HETEROZYGOUS FOR THE ARYL HYDROCARBON RECEPTOR.

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Gestational and lactational exposure to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been reported to alter spatial learning and memory in rats tested on a radial arm maze (RAM). TCDD is believed to exert most of its effects through binding to the aryl hydrocarbon receptor (AhR). To determine if TCDD-induced alterations in spatial learning are mediated by the AhR, we tested male and female AhR knockout ($AhR^{-/-}$), heterozygous ($AhR^{+/-}$), and wild-type ($AhR^{+/+}$) mice on the 8-arm RAM. Following testing on the maze we examined hippocampal morphology using the Timm's silver-sulfide stain. This stain permits accurate measurement of the intra- and infrapyramidal mossy-fiber field (IIP-MF), the area of which has been reported to correlate with RAM learning. $AhR^{+/-}$ males and females were timemated, the dams were dosed with 0 or 5 µg TCDD/kg body weight on day 13 of gestation and the pups were weaned on postnatal day 21. Upon maturation, male and female $AhR^{+/+}$, $AhR^{+/-}$, and $AhR^{-/-}$ mice from TCDD-exposed and unexposed litters were tested on the RAM. Following testing their brains were processed for staining. TCDD-exposed female $AhR^{+/-}$ mice made significantly more errors on the RAM than their respective controls and exhibited a decrease in the relative size of the IIP-MF. None of the other TCDD-exposed groups differed from their respective controls with regard to maze performance or hippocampal morphology. The reduction in IIP-MF area provides a morphological basis for the learning deficit that was observed in the female $AhR^{+/-}$ mice. The role of the AhR remains unclear, but the data suggest that the amount of receptor and sex of the animal interact in some way. Possibly, the AhR interacts with hippocampal steroid or glucocorticoid receptors and/or their pathways. (Supported by NIH Grant ES01332)

642 EVIDENCE THAT GABAERGIC NEURONS OF THE PREOPTIC AREA OF THE BRAIN ARE TARGETS OF TCDD REPRODUCTIVE TOXICITY IN ADULT AND PERINATAL RATS.

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Acute or chronic exposure to the arylhydrocarbon receptor (AhR) ligand, 2, 3, 7, 8-tetrachlor-*p*-dibenzodioxin (TCDD), in adulthood or during development interferes with reproductive functions. However, it has been debated whether TCDD exerts these effects directly by binding to the AhR in specific brain regions, or secondarily through effects on liver or peripheral tissues. We previously found that, in the rat, mRNAs encoding arylhydrocarbon receptor (AhR), as well as its companion proteins, arylhydrocarbon receptor nuclear translocator (ARNT) and ARNT2 are present in a number of regions important for reproductive function. In the present studies, we used *in situ* hybridization histochemistry (ISHH) to map these genes in perinatal rat brains and found that they were expressed in the same regions as in adults. Of particular importance, we found them expressed in the anteroventral periventricular/median preoptic nucleus (AVPV/MPN) region, a region known to be sexually dimorphic and to play a critical role in estradiol-dependent preovulatory luteinizing hormone (LH) surge release in females. GABAergic neurons in this region have estrogen receptors (ER) and are important for the generation of the LH surge. Therefore, we next used dual-label ISHH and found that glutamate decarboxylase (GAD; a marker of GABAergic neurons) and AhR genes are expressed in the same cells in the AVPV/MPN region, as well as throughout the brain in both adults and perinates. Furthermore, we found that a single exposure to 1 microgram/kg of TCDD on gestational day 15 and through lactation abolished sexual differences in GAD gene expression in the AVPV/MPN region in animals examined at postnatal day 3. These neuroanatomical findings support the hypothesis that GABAergic neurons in the preoptico-hypothalamic region mediate the developmental and adult effects of TCDD on fertility.

643 DEVELOPMENTAL EXPOSURE TO A BROMINATED FLAME-RETARDANT, 2, 2', 4, 4', 5, 5'-HEXABROMO-DIPHENYL ETHER (PBDE 153) AFFECTS BEHAVIOUR AND CHOLINERGIC NICOTINIC RECEPTORS IN BRAIN OF ADULT MICE.

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Flame-retardants are used to suppress or inhibit combustion processes in order to reduce the risk of fire. One class of flame-retardants, polybrominated diphenyl ethers (PBDEs), have been found to increase in the environment and in human mother's milk. In recent studies we have seen that neonatal exposure to some brominated flame-retardants, such as PBDE 47, PBDE 99 and PBDE 209, can cause permanent aberrations in spontaneous behaviour, an effect that worsen with age. PBDE 99 and PBDE 209 have also been shown to induce these effects during a defined critical phase of neonatal brain development. In view of an increasing amount of PBDEs in mother's milk and in the environment, the present study was undertaken to investigate whether PBDE 153 can induce persistent neurotoxic effects on spontaneous behaviour, learning and memory and cholinergic nicotinic receptors in mice. Mice were exposed to 0.45, 0.9 or 9.0 mg PBDE 153/kg body weight on postnatal day 10. Spontaneous behaviour was observed in 2-, 4- and 6-months-old mice, and mice exposed to 0.9 or 9.0 mg PBDE 153/kg body weight on postnatal day 10 showed significantly impaired spontaneous motor behaviour at all three testing occasions. This effect worsened with age. At 6 month of age learning and memory was assessed in a Swim maze of Morris water maze type. The ability to locate the platform was significantly impaired in animals neonatally exposed to 0.9 or 9.0 mg PBDE 153/kg body weight, compared to control animals. The mice were sacrificed at 6 months of age and analyzed for cholinergic nicotinic receptors in hippocampus using α-Bungarotoxin. There was a significant decrease in the density of specific [^{3}H]-α-Bungarotoxin binding sites in the hippocampus in mice exposed neonatally to 9.0 mg PBDE 153/kg body weight. In conclusion, neonatal exposure to PBDE 153 induces behavioural derangements in adult mice as well as defects learning and memory. In addition, the cholinergic nicotinic receptors in hippocampus were affected.

644 DEVELOPMENTAL EXPOSURE TO POLYBROMINATED DIPHENYL ETHERS DOES NOT ALTER SYNAPTIC TRANSMISSION OR LTP IN HIPPOCAMPUS.

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Polybrominated diphenyl ether (PDE) flame retardants bioaccumulate in the environment, in wildlife, and in humans. Concern has been raised over potential thyrotoxic effects of this class of xenobiotics. Severe hypothyroidism during critical periods of brain development leads to alterations in hippocampal structure and physiology and produces learning deficits. The present work examined field potentials evoked in the dentate gyrus of adult offspring of dams treated daily with 0 or 30 mg/kg of DE71, a commercial PDE mixture, from gestational day 6 to weaning at postnatal day (PND)21. This regimen produced moderate decreases (~55%)in thyroxine (T4) during the early postnatal period that returned to control levels by PND36. Triiodothyronine (T3) was unaffected. Male offspring(4-8 mon)were anesthetized with urethane and electrodes implanted in the perforant path and dentate gyrus. Input/output (I/O) functions were collected to assess the integrity of synaptic transmission. Long-term potentiation (LTP) was induced by delivering theta burst stimulation at a modest stimulus intensity of 300µA and I/O functions were re-evaluated 1 hr later. Thereafter, a second set of trains at maximal stimulus intensity (1500 µA) was administered and a third I/O collected 15 min later. No alterations in population spike, EPSP slope, or postsynaptic potential amplitudes were observed in baseline I/O recordings of DE71-treated animals relative to controls. Modest LTP was observed in both groups in response to the low intensity train stimulation, and robust LTP was induced by the higher intensity train series. No group differences in LTP magnitude were revealed. The lack of effects of PDE differs from the response to either propylthiouracil or A1254, agents that also transiently reduce thyroid hormones during development, albeit it to a greater degree. These data suggest that the modest reductions in T4 induced by this dosing regimen with DE71 were not sufficient to alter synaptic transmission in the dentate gyrus of the hippocampus. *(Does not reflect USEPA policy).*

645 INTERACTIVE EFFECTS OF METHYLMERCURY AND PCB MIXTURES ON NEURODEVELOPMENT IN MICE.

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To examine the effects of methylmercury (MeHg) and PCB congeners either alone or in combination on neurodevelopment, several neuronal proteins were measured in the neonatal mice at different time points. From gestation day 10 until postnatal

day (PND) 21, each group of pregnant C57BL/6 mice were exposed to control, methylmercury alone (40 ug/kg), PCB 153 alone (5 mg/kg), PCB 126 alone (5 ug/kg), the mixture of PCB 153 (5 mg/kg) and PCB 126 (5 ug/kg), the mixture of PCB 153 (5 mg/kg) and methylmercury (40 ug/kg), and the mixture of all chemicals. The mortality rate of neonatal mice in the group treated by MeHg alone was about 70 %, whereas those in the other mixture group (the mixture of MeHg and PCB 153 and the mixture of all chemicals) was under 10% suggesting antagonism toward MeHg toxicity. At PND 9, 16, 23, and 30, brains of neonatal mice from each group were collected and dissected into cerebellum, hippocampus, and cerebral cortex. Western blot analysis was performed to measure CREB, ERK 1/2, PKC, CaMK II, and CaMK IV in each brain regions. Preliminary results showed that the expression of CREB in cerebellum of the group exposed to the mixture of all chemicals increased four times higher than control at PND 9 but decreased dramatically after that, resulting in the 50 % decrease comparing with control at PND 30. The expression of CREB in hippocampus showed similar pattern, but the extent of differences in the expression was smaller than in cerebellum. The expression of CaMK IV in hippocampus of the group exposed to the mixture of all chemicals decreased comparing with control at PND 9. However, the expression of CaMK IV in cerebellum of the group exposed to the mixture of all chemicals increased comparing with control at PND. These results suggest that repeated exposure to methylmercury and PCBs *via* gestational and lactational transfer affects normal expression of neuronal proteins in a non-additive manner. (Supported by NIEHS R03 ES10116)

646 PERINATAL EXPOSURE TO A POLYBROMINATED DIPHENYL ETHER MIXTURE (DE-71) DISRUPTS THYROID HORMONES BUT NOT NEUROBEHAVIORAL DEVELOPMENT.

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Polybrominated diphenyl ethers (PBDEs), produced commercially as mixtures, are used as flame-retardants for numerous consumer products. Because of their lipophilicity and persistence, PBDEs have become ubiquitous environmental contaminants. Previous work in our Lab. has demonstrated that short-term (4 day) *in vivo* exposure to DE-71 in young rats (a commercial polybrominated diphenyl ether mixture containing mostly tetra- and penta-bromodiphenyl ethers) induces hypothyroxinemia. In this study, motor and auditory function were measured in offspring following perinatal maternal exposure to DE-71. Primiparous rats were orally administered DE-71 (0, 1, 10 and 30mg/kg/day) in corn oil from gestation day 6 (GD6) to postnatal day 21 (PND21). Offspring were evaluated at various ages for body weight, thyroid hormones, hepatic enzymes, eye opening, survival, motor activity development, and auditory startle response. There was no effect of DE-71 on body weight gain or survival in either the dams or offspring. There were dose-dependent decreases in thyroxine (maximal decrease of 55% on PND14), and increases in hepatic glucuronidation (3-fold). Both endpoints return to control levels by PND36. There were normal age-dependent changes in motor and sensory behaviors, but no effects of treatment. Benchmark dose (BMD) modeling resulted in BMDs of 2.3 and 5.5 mg/kg/day, and BMDLs (lower bound) of 0.9 and 3.4 mg/kg/day, for serum T4 and hepatic glucuronidation, respectively. These data suggest that DE-71 alters thyroid homeostasis during development, but does not seem to alter sensory or motor development as determined by these endpoints. *This abstract does not necessarily reflect USEPA policy.*

647 LONG-LASTING NEUROSTRUCTURAL CONSEQUENCES IN THE RAT HIPPOCAMPUS BY DEVELOPMENTAL EXPOSURE TO A MIXTURE OF POLYCHLORINATED BIPHENYLS.

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The objective of the study was to assess the effects of developmental exposure to a commercial mixture of PCBs (Aroclor 1254) on neuronal dendritic morphology of hippocampal CA1 pyramidal neurons in postnatal day (PND) 22 and PND 60 male Long-Evans rats. Rat pups were born to mothers who were exposed to Aroclor 1254 (AccuStandard Inc.., Lot # 124-191; 0 and 6 mg/kg/day) from gestational day 6 through PND 21. Thus, pups were exposed to PCBs *in utero* and through weaning. Male rats (N = 5-6 per group) were sacrificed on PND 22 and PND 60. Brains were formalin-fixed for Rapid Golgi staining of tissue blocks; coded slides of hippocampus were prepared. For branching and spine analysis, 6 CA1 pyramids were randomly selected from each brain. Camera lucida drawings of the basilar dendritic tree were analyzed using the Sholl method of concentric circles. For spine analysis, counts were made along internal and terminal tip segments of 6-7 neurons from each brain. Results of the branching analysis for the PND 22 PCB-exposed rats showed that, compared to controls, there was significantly less dendritic branching in the outer 2/3rds of the dendritic tree ($p = 0.002$, Wilcoxon test). Spine analysis also showed a reduction in spines on the terminal tips segments of 22 day old PCB-exposed rats ($p=0.005$, T-test). These results suggest that perinatal exposure to Aroclor 1254 resulted in morphometric changes in hippocampus. By PND 60, spine density on terminal tip segments had returned to normal levels. However, branching analysis now showed that compared to controls there was an excessive amount of dendritic material in the distal 2/3rds of the tree ($p=0.001$, Wilcoxon test). This suggested a possible structural "hyperplasticity" in neurons damaged by PCB exposure during the developmental period with a residual long-term dysmorphic impact on hippocampal circuitry. (This abstract does not necessarily reflect USEPA policy).

648 DEVELOPMENTAL NEUROTOXICITY (DNT) STUDIES WITH TRIBUFOS (DEF) AND METHAMIDOPHOS.

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DNT studies (OPPTS 870.6300) were conducted with two organophosphorus (OP) compounds in response to a data call-in notice issued by the USEPA. Each OP was administered *via* the diet at 4, 40 and 200 ppm for DEF and 1, 10 and 30 ppm for methamidophos to mated female Wister rats (n=20/level) from gestation day (GD) 0 through postnatal day (PND) 21 for the offspring. The offspring were evaluated for clinical signs, weight gain, sexual maturation, motor activity (figure-8 maze), auditory startle habituation, associative learning and memory (M-maze and passive avoidance), neuropathology and brain measurements. Whole-brain, erythrocyte and plasma cholinesterase (ChE) activities were measured in the dams on LD 21 and in the pups on PND 4 or 11 and again on PND 21. Slight ChE inhibition in the dam was the only effect at 40 ppm with DEF and 1 ppm with methamidophos. In pups, ChE inhibition occurred at the mid- and high-dietary levels of methamidophos and at the high dose only with DEF. Effects in pups at the higher doses included decreased weight gain and decreased motor activity during treatment, a reduced startle response on PND 22 and delays in vaginal patency (both OPs) and preputial separation (DEF only). With the high dose of DEF, absolute (but not relative) brain weight was reduced on PND 11 but not on PND 21 or 70. The only effect that persisted to term on PND 70-75 was a lower body weight at the high dose with DEF. Measures of cognition and habituation (motor activity and startle) were not affected and neuropathology was not evident with either OP at any dietary level. The overall NOELs in the dam and offspring were 4 ppm and 40 ppm, respectively, for DEF and <1 ppm and 1 ppm, respectively, for methamidophos. The overall NOEL in each study was based on inhibition of ChE activity in the dam. This outcome and the resulting overall NOELs are consistent with those of the two-generation reproduction studies.

649 CONTROL BEHAVIORAL DATA FROM DEVELOPMENTAL NEUROTOXICITY STUDIES (DNT)AND PERI-POSTNATAL STUDIES: IS SEXUAL MATURATION IN DNT STUDIES IMPROVED BY ANALYZING THREE PUP/SEX/LITTER?

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Behavioral evaluations of the pup and adult rat (motor activity, acoustic startle habituation, sexual maturity) are requirements of DNT studies conducted under USEPA guidelines (OPPTS 870.6300). Learning and memory (i.e. passive avoidance and watermaze) and sexual maturity have been conducted as part of peri-postnatal studies since the mid-80s for pharmaceuticals. Relatively few DNT studies have been conducted to date, and the ranges of normal values for the various parameters have not been previously established. The behavioral values for control rats participating in nominally identical DNT and approximately 60 pharmaceutical testing studies have been compared. The results of approximately 40 studies were presented in 2000. Pregnant Sprague-Dawley rats were administered control substances orally beginning on Day 0 or 6 of gestation and continuing through Day 11 or 21/22 of lactation [Day of birth = DP 1]. Rats were tested for motor activity on DPs 14, 18, 22 and 61"2. Rats were tested for passive avoidance on DPs 24"1 and then retested one week later. Rats were tested for auditory startle habituation on DPs 23 and 61"2. Rats were tested in a watermaze on DPs 61"2 and retested one week later. Rats were evaluated for sexual maturity (female rats beginning on DP 28 and male rats beginning on DP 39). The availability of such control data for behavioral measurements will help clarify the extent to which between subject and between litter (sexual maturation) variation affects the statistical power of comparisons between treatment and control groups in DNT studies.

650 NEUROMORPHOLOGICAL EVALUATION OF CONTROL DATS FROM SEVEN DEVELOPMENTAL NEUROTOXICITY STUDIES (DNT)INCLUDING POSTIVE CONTROL DATA.

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Quantitative morphological measurements of the pup and adult rat brain are a requirement of DNT studies conducted under USEPA guidelines (OPPTS 870.6300). Relatively few guideline studies of this type have been conducted to date, and the range of normal values for the various parameters has not been previously established. The morphometric values for control rats participating in seven nominally identical DNT studies will be compared. Pregnant Sprague-Dawley rats were administered control substances orally beginning on Day 0 or 6 of gestation and continuing through Day 11 or 21/22 of lactation [Day of birth = DP 1]. Brains from DP 12 pups and adult rats were weighed, and the gross anterior-posterior lengths of the cerebrum and cerebellum were recorded. Microscopic measurements of the frontal cortex, parietal cortex, hippocampal gyrus, corpus callosum and cerebellum were performed and recorded. Qualitative neuropathological evaluations were performed on the DP 12 rat brain and the adult rat brain, spinal cord, dorsal root ganglia, Gasserian ganglia and associated trigeminal nerve, and selected peripheral nerves. Morphometric measurement data, collected during a validation study, comparing Day 10 and 12 Sprague-Dawley rat pups are provided. The results demonstrate that the measurement data collected from the selected neuroanatomic areas reveal age-related developmental differences that might, therefore, be predictive of either an *in utero* neurotoxic effect or a delay in brain development. The availability of control as well as positive control data for morphometric measurements will help clarify the extent to which between-subject variation affects the statistical power of comparisons between treatment and control groups in DNT studies.

651 THE RELIABILITY AND SENSITIVITY OF HISTOMORPHOMETRIC METHODS IN DETECTING MORPHOLOGICAL CHANGES IN THE DEVELOPING RAT BRAIN.

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In regulatory developmental neurotoxicity studies the study guidelines require that morphometric analyses be conducted on the developing rat brain. Histomorphometric evaluation is recognized as a useful tool to quantify gross changes and cellular migration in the developing brain; however, it is important to identify and examine regions of the brain that are sensitive to toxicological insult. Further, high quality sections and consistent histomorphometric techniques are essential for reliable quantitative data. The developing rat brain undergoes rapid growth during the first 2 weeks after birth and morphometric changes would be anticipated during this time. The objective of the present study was to ensure that the histomorphometric procedures employed in our laboratory are sensitive enough to detect the subtle morphological changes occurring in the developing brain. For this we compared the morphometry of various brain regions from 10 day and 13 day old rats. The brains (6/sex/age) were immersion fixed in 10% neutral buffered formalin, embedded in paraffin, and stained. Histomorphometric evaluations were made by the same operator using a BIOQUANT/TCW image analysis system (BIOQUANT-R&M Biometrics, Inc..) on one section of forebrain (left and right frontal cortex; left and right parietal cortex; corpus callosum) and cerebellum (lobule base thickness; internal and external granular layer thickness) from each animal. Significant differences between 10 and 13 day old pups were detected in the frontal and parietal cortex (males only), the thickness of the base of cerebellar lobule 6/7/8 and lobule 9 (females only), and in the internal and external granular cell layers of the cerebellum (males and females). Our results indicate that during the second week of postnatal development the internal and external granular layers of the cerebellum are the most sensitive and consistent brain regions that can be measured reliably.

652 DEVELOPMENTAL NEUROTOXICITY TESTING GUIDELINES: A QUALITATIVE RETROSPECTIVE ANALYSIS OF POSITIVE CONTROL DATA.

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The USEPA Developmental Neurotoxicity (DNT) Study Test Guideline calls for both functional and neuropathological assessments in offspring during and following maternal exposure. This guideline also requires data from positive control (PC) agents. Submission of these data permit evaluation of laboratory proficiency in the detection of changes in the structure and function of the developing nervous system and comparison of the sensitivity of assessments in different studies and labs. This project surveyed approaches taken in contract and industrial laboratories in generating and providing these data. Data from 24 DNT studies from 13 different laboratories were summarized by method, e.g., motor activity, for information on: age relevance, Dose-Response, gender, group size, statistics, report quality, quality assurance, age of data relative to main study, and relevance of methods to the main study. Endpoints included: developmental landmarks, FOB, motor activity, startle response, learning and memory, standard histopathology and brain morphometry. Results ranged from no PC data for two laboratories, to one laboratory which submitted 16 different reports. The qualitative range was similarly broad, from excellent to extremely poor. Various quality problems were identified including: poor report structure (e.g., copies of poster presentations), lack of individual data, inadequate methodological details, differences in methods between the PC report and the main study, submission of very old data or data from completely different laboratories, significant variance in the submitted data, use of inappropriate PC chemicals or doses that were without effect, lack of statistical analysis, use of only one sex, and use of inappropriate age animals. Preliminary analyses suggest that there were only 4 out of 13 laboratories judged to have submitted adequate PC data. *This abstract does not necessarily reflect the policy of the USEPA*

653 PRENATAL NICOTINE EXPOSURE EVOKES LONG-TERM ALTERATIONS OF CELL STRUCTURE IN HIPPOCAMPUS AND SOMATOSENSORY CORTEX.

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Offspring of women who smoke during pregnancy show behavioral abnormalities including increased incidence of attentional deficit, learning disabilities and cognitive dysfunction. Animals models indicate that nicotine elicits changes in neural cell replication and differentiation, leading to deficits in synaptic neurochemistry and behavioral performance, many of which first emerge at adolescence. In this study, we evaluated cellular morphology and regional architecture in the juvenile and adolescent hippocampus and the somatosensory cortex in rats exposed to nicotine prenatally. Pregnant rats were given nicotine throughout gestation *via* minipump infusion of 2 mg/kg/day, a regimen that elicits nicotine plasma levels comparable to those found in smokers. On postnatal days 21 and 30, brains were perfusion-fixed, coronal slices were taken between the anterior commissure and median eminence, and the morphology of the dorsal hippocampus and somatosensory cortex were characterized. In the hippocampal CA3 region and dentate gyrus, we found a substantial decrease in cell size, with corresponding decrements in cell layer thickness, and increments in cell packing density. Smaller, transient changes were seen in CA1. In layer 5 of the somatosensory cortex, although there was no significant decrement in the average cell size, there was a reduction in the proportion of medium-sized pyramidal neurons and an increase in the proportion of smaller, nonpyramidal cells. Taken together with previous work on neurochemical and functional defects, these data demonstrate that prenatal nicotine exposure compromises neuronal maturation, leading to long-lasting alterations in the structure of key brain regions involved in cognition, learning and memory. (Supported by USPHS DA14247 and by a fellowship from IBRO)

654 NICOTINE AFFECTS ZEBRAFISH MOTONEURON DEVELOPMENT.

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Nicotine is a drug of abuse that has a profound impact on society. Of great concern is the harm that is caused when a pregnant mother smokes and exposes her unborn child to nicotine. Nicotine has been shown to be a developmental toxicant in several experimental models, but the mechanism by which nicotine alters development is not well understood. It is thought that the actions of nicotine are mediated by the nicotinic acetylcholinergic receptors (nAChRs). We are utilizing zebrafish embryos to gain insight into how nicotine modulates development with a particular emphasis on nervous system development. For these studies, zebrafish embryos were exposed to 33 micromolar nicotine beginning at 22 hours post-fertilization (hpf). At this dose, embryos do not respond to touch (at 48hpf) and are functionally paralyzed. At 48 hpf, nicotine had no apparent effect on the gross morphology of the embryo. However at 66 hpf and 120 hpf, embryos exposed to nicotine were 6% and 7% shorter in length, respectively, compared to controls. The morphological deficit coupled with the behavioral deficit suggested to us that either motoneuron or neuromusculature development was altered by nicotine. In order to investigate these possibilities, we imaged living transgenic zebrafish that express green fluorescent protein (GFP) in their spinal secondary motoneurons. Embryos exposed to

nicotine had significantly fewer GFP positive neurons than stage matched controls. This effect was easily detectable at 54-60 hpf. This effect was partially reversed by 2 mm MLA, a nicotinic antagonist known to act at the alpha 7 receptor subtype. Furthermore, 20 mm DHβE, an alpha 4/beta 2 subtype specific nicotinic receptor antagonist also reversed the nicotine effects. These results demonstrate that zebrafish is an excellent vertebrate model to study nicotine developmental toxicity, and suggest that an "alpha4/bera 2 like" nAChR subtype may be involved in mediating nicotine's actions in zebrafish early life stages.

655 PHARMACOKINETICS OF SB-247083, A POTENT AND SELECTIVE ENDOTHELIN-A RECEPTOR ANTAGONIST, IN THE RAT, DOG, AND MONKEY.

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The endothelins (ET) are among the most potent vasoconstrictors identified to date, and have been implicated in such diseases as renal failure, pulmonary hypertension, atherosclerosis, and congestive heart failure. There is currently interest in developing selective antagonists of the ET-A subtype receptor, and one such antagonist is SB-247083 ((E)-[1-butyl-5-[2-(2-carboxyphenyl) methoxy-4-chlorophenyl]-1H-pyrazole-4-yl]-2-[5-methoxydihydrobenzofuran-6-yl]methyl]-2-propionic acid). This investigation was conducted to evaluate the preclinical pharmacokinetics of SB-247083. Clearance of SB-247083 was low in the rat, moderate in the monkey, and high in the dog. Clearance was linear in the rat, with modest evidence of nonlinear elimination at high concentrations in the dog and monkey. First-pass hepatic extraction was approximately 60% in the rat, and ranged from 50 to 90% in the monkey. Oral bioavailability of SB-247083 administered as a solid formulation of the free acid is good in the rat (24%), but low in the dog (1 to 8%) and the monkey (2%). Pre-administration of 0.1 N HCl to normalize the achlorhydric fasting dog stomach did not improve bioavailability. Various formulations and salt forms of SB-247083 were evaluated; the most favorable was formulation as the monoarginyl salt, which approximately doubled oral bioavailability in each species tested. These data suggest that SB-247083 may be a promising ET-A receptor antagonist, and may have utility in various ET-mediated diseases.

656 KINETICS AND LOCOMOTOR ACTIVITY FOLLOWING COCAINE AND KETAMINE ADMINISTRATION IN RAT.

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The co-abuse of cocaine (COC) and ketamine (KET) occurs with high frequency. The presence of another active substance with COC allows the potential of various drug-drug interactions to occur. COC metabolism may play an important role in its toxicity. Benzoylecgonine (BE) has been reported to be a potent vasoconstrictor and convulsant agent. This study investigated the plasma time course, tissue distribution and locomotor activity of COC or KET alone and their combination in rat. COC (5 mg/kg i.v.), KET (100 mg/kg p.o.) or KET followed by COC (same doses and routes of administration) were utilized. KET administered concurrently with COC, significantly reduced COC area under the curve (AUC) and elimination half-life, while the total clearance was increased *versus* COC administration alone. The AUC of plasma BE was significantly increased in the combination treatment compared to COC alone. Tissue distribution study revealed that, the brain, heart and liver contents of COC and norcocaine were significantly lowered at 5, 15 and 30 min following KET administration *versus* COC alone. However in the same organs, BE was significantly higher in the combination group compared to COC alone. On the other hand, COC treatment did not affect the kinetics of KET as a parent compound. Locomotor activity study showed that, rats treated with COC and KET in combination had a significantly lower horizontal locomotor activity compared to COC treatment. The results suggest that KET increases the metabolism of COC sufficiently to alter its behavioral effects. The high plasma and tissue concentrations of BE after the combination treatment, which is potent cardiovascular and neurotoxic agent, may possibly affect the toxicological profile of COC as well. (Supported in part by the Egyptian government).

657 PLASMA PHARMACOKINETICS OF TRICLOSAN FOLLOWING REPEATED DERMAL EXPOSURE.

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Triclosan (TCS: 2, 4, 4'-trichloro- 2'- hydroxydiphenyl ether) is an anti-microbial agent that has been used for over 30 years in a variety of personal care products. This study evaluated the steady-state plasma concentration and pharmacokinetic profile of TCS following repeated dermal application. Plasma levels of TCS were followed in 13 human volunteers (7 males and 6 females) after use of a commercial hand wash containing 1% TCS, 6 times per day for 20 days. Volunteers were instructed not to use any other TCS-containing products during the course of the study. Blood samples were taken prior to first hand washing on study day 1 (baseline TCS level) and study days 5, 10, 15, and 20. Blood samples were also taken post exposure on study days 21, 23 and 30. Samples were then analyzed by LC/MS/MS for free and total (conjugated) TCS in plasma. On day 20, the subjects were sequestered for 12 hours, during which hand washing was continued every 2 hours and blood samples obtained prior to each hand wash. Pharmacokinetic analysis of the plasma concentration data supports a first order single compartment model for TCS. Steady state levels of free and total TCS were reached by study day 5. Mean (±SD) steady state levels of free and total TCS from day 5 to day 20 ranged from 5.4(±5.0) to 10.6(±11.6) ng/ml and 67.1(±34.7) to 74.9(±48.9) ng/ml, respectively. Maximum free and total TCS plasma levels during the study for any volunteer were 69.9 and 229 ng/ml. The average elimination half life and eliminate rate constant, calculated from the individual values for the study population, were 1.4 days and 0.615 day^{-1}. Overall, the study results show that TCS is absorbed through the skin and rapidly eliminated from the body. Furthermore, the data support that human exposure to TCS resulting from continued use of a hand wash containing 1% TCS is significantly below exposures associated with animal toxicity end points.

658 APPLICATION OF THE CULEX™ AUTOMATED BLOOD SAMPLER IN THE PHARMACOKINETIC STUDY OF SU006668 IN AWAKE RATS.

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Conventionally in the *in vivo* rat study of pharmacokinetics, a series of blood samples are collected manually through a dwelling intravenous catheter. A novel robotic device, Culex™ automated blood sampler (Bioanalytical Systems, Inc.., West Lafyette, IN), provides an alternative way to automatically collect blood from awake and freely moving rats. The purpose of this study was to evaluate the feasibility of the Culex™ automated blood sampler in the application of pharmacokinetic studies in rats. SU006668, an antiangiogenetic agent in Phase I clinical development for the treatment of solid tumors, was studied as a model drug for this purpose. Fifteen rats received intravenous (IV, n=7) or oral (PO, n=8) administration of the drug. For each group, the blood samples were collected up to 12 hours either by using manual blood collection method (n=4 for both IV and PO groups) or by using Culex™ automated blood sampler (n=3 for IV and n=4 for PO). The plasma concentrations of SU006668 were determined using a LC-MS/MS method. The pharmacokinetic parameters were determined by non-compartmental analysis using Winnonlin Professional Edition. The results shown below indicate that the pharmacokinetic parameters obtained from two blood sampling techniques (Culex vs Manual) were comparable (P>0.05, t-test), and consistent with the historical pharmacokinetic data on SU006668. The study suggests the feasibility of blood sampling by the Culex system for the study of pharmacokinetics.

	IV, Manual	IV, Culex	PO, Manual	PO, Culex
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
CL (mL/min/kg)	6.7 ± 1.1	6.8 ± 1.0		
V_d (L/kg)	1.0 ± 0.2	1.3 ± 0.2		
AUC (hr*ng/mL)	25172 ± 3882	25005 ± 3872	29656 ± 2453	27568 ± 9368
$T_{1/2}$ (hr)	1.8 ± 0.5	2.3 ± 0.3	2.2 ± 0.9	1.6 ± 0.3
T_{max} (hr)			2.4 ± 0.8	2.1 ± 0.3
C_{max} (ng/mL)			12738 ± 2052	12645 ± 6149
F (%)			59 ± 5	55 ± 19

659 CORTICOSTERONE IN DRINKING WATER ENHANCED THE ABSORPTION OF A SINGLE ORAL DOSE OF CORTICOSTERONE.

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Corticosteroids are stress indicators that contribute to many body functions. Effects of corticosterone exposure on corticosterone kinetics were studied in adult male Long Evans rats. Some of these were gavaged with corticosterone in corn oil at 20

mg/kg body weight (n = 4). The control rats were given only corn oil (n = 4). Each rat was bled at 0, 15, 30, 60, 120, 240, 480, and 720 minutes after the treatments. Plasma corticosterone levels rose to a peak level of 713 ± 59 ng/ml by 30 minutes after dosing. Plasma corticosterone gradually decreased until 240 minutes, when the level was similar to that of the controls. After 12 days without treatment, the rats were given either 2.5% ethanol or corticosterone 400 ug/ml in drinking water for 29 days. Ethanol 2.5% in drinking water increased the basal corticosterone levels. The corticosterone-drinking rats were then gavaged with corticosterone 20 mg/kg. Blood samples were taken as noted above. The plasma corticosterone levels of the corticosterone-drinking water rats (106 ± 71 ng/ml) were lower than the ethanol group (453 ± 71 ng/ml)), and also lower than the levels in these rats before they drank the corticosterone water (227 ± 59 ng/ml). The peak plasma corticosterone after gavage was 975 ± 71 ng/ml at 15 minutes. The corticosterone in drinking water, therefore, enhanced the absorption of a single oral corticosterone dose. This demonstrates that corticosterone in drinking water can affect corticosterone kinetics. (Supported by DAMD 17-99-1-9489.This abstract does not necessarily reflect the position or policy of the US Government).

660 CONTINUOUS INFUSION IN MARMOSET MONKEYS: FEASIBILITY OF A PORT-CATHETER TECHNIQUE.

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The common Marmoset (Callithrix jacchus jacchus) can play an important role as the second species in the safety evaluation of new drugs. Hence the need occurred to evaluate the suitability of this primate as a model for continuous infusion studies. This work investigates the feasibility of a port-catheter technique in marmosets and concentrates on the technical details of the surgical implantation procedure.The right and the left thigh as well as the abdominal wall and the dorsal region of the thorax are shaved. The operation area in the upper field of the right thigh is sterilized with Betaisadona solution. Following intramuscular premedication with Ketamine, Xylazine and Atropine, anaesthesia of the marmoset is performed by inhalation narcosis with Isoflurane. During the operation process, breathing is supported using a respirator.The proximal section of the V. femoralis dexter is selected for accessing to the venous system. The silicone catheter (3 French O.D.) is placed close to the V. renalis sin., and the disconnected distal V. femoralis is used as a point of fixation. A subcutaneous pocket in the region of the upper thigh is used to connect the externally fixed titanium port (Type POPS II), placed on the animals's back, with the injection site. The external port is fixed on the skin by a patch, the silicone membrane of the port is directed cranially to inject substances easily. To protect the surgical operation area and to support wound healing, a tube-shaped wound cover is used to enclose thorax and abdominal body. The suture material is removed after 8 days. A heparine solution of 5 IU/mL is regularly used to rinse and protect the infusion system.In conclusion, a port-catheter system is feasible for marmoset monkeys since it is shown here that such a system can successfully be implanted in these very small non-human primates. First experiences confirm successful and intermittent use of the system for more than six weeks.

661 INCREASED PLASMA LEVEL OF ACETAMINOPHEN-GLUCURONIDE IS ASSOCIATED WITH INDUCTION OF MULTIDRUG RESISTANCE PROTEIN 3 (MRP3) IN LIVER.

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Previous studies report that treatment with the cytochrome P450 2B1/2 inducers phenobarbital (PB) and trans-stilbene oxide (TSO) can decrease biliary excretion of acetaminophen-glucuronide (AA-GLUC) and increase excretion of AA-GLUC into blood. The canalicular Multidrug Resistance Protein 2 (Mrp2) can transport glucuronide conjugates into bile, whereas the sinusoidal protein Mrp3 is thought to transport these conjugates into blood. Hence, it is hypothesized that Mrp2 and Mrp3 are responsible for the excretion of AA-GLUC conjugate into bile and blood, respectively. Thus, PB and TSO-induced alterations in vectoral excretion of AA-GLUC may occur *via* increased hepatic Mrp3 levels. Therefore, the goal of this study was to determine 1) whether PB and TSO, compounds known to increase plasma concentrations of AA-GLUC up-regulate Mrp3 expression, and 2) whether compounds that induce Mrp3 expression, such as diallyl sulfide (DAS) and oltipraz (OLT), can correspondingly decrease biliary and increase hepatovascular excretion of AA-GLUC. Sprague-Dawley rats were administered PB, TSO, OLT, or DAS, or vehicle for four days. To determine the effects of these chemicals on AA excretion, the carotid artery and bile duct were cannulated and blood and bile were collected at various intervals up to 90 min after AA injection into the carotid cannula. AA-GLUC content in bile and blood was quantified by HPLC and Mrp2 and Mrp3 mRNA was quantified using the branched DNA signal amplification assay. Interestingly, all of the chemicals increased the hepatovascular and decreased the biliary excretion of AA-GLUC. Furthermore, all of the compounds dramatically increased hepatic Mrp3 mRNA by at least 6 fold, but had little or no effect on hepatic Mrp2 mRNA levels. Together, these studies correlate increased Mrp3 mRNA levels with increased hepatovascular excretion of AA-GLUC and suggest that induction of Mrp3 affects the route of drug excretion. (Supported by NIH grants ES-09716 and ES-07079)

662 KINETICS OF PROPYLENE GLYCOL MONOMETHYL ETHER (PGME) AND PGME ACETATE (PGMEA) FOLLOWING INTRAVENOUS (IV) ADMINISTRATION TO MALE FISCHER 344 RATS AND THE *IN VITRO* HYDROLYSIS OF PGMEA IN RAT AND HUMAN BLOOD AND LIVER HOMOGENATE.

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The blood kinetics of PGMEA and PGME following iv administration to rats was conducted to determine toxicological equivalency between the two materials. The *in vitro* rates of hydrolysis of PGMEA to PGME in incubations with rat and human blood as well as liver homogenates were measured for use in a future PB-PK model for PGME. For investigation of the kinetics of PGME or PGMEA following iv administration to rats, equimolar doses of 10 mg PGME/kg or 14.7 mg PGMEA/kg and 100 mg PGME or 147 mg PGMEA/kg were used. Blood samples were drawn from the jugular vein cannula through 12 h post-dosing and analyzed for PGMEA and PGME. Plasma time course of PGME following iv administration of 10 mg PGME/kg or 14.7 mg PGMEA/kg were identical; similarly for the high dose of 100 mg PGME/kg or 147 mg PGMEA/kg. Plasma time courses of PGMEA following iv administration of 14.7 or 147 mg PGMEA/kg bw were determined and half-lives were 1.55 and 3.37 min, respectively. Incubations were conducted through 6 h at concentrations of 5 and 50 µg PGMEA/ml. Samples were analyzed *via* PCI or EI-GC/MS for determination of PGMEA and PGME in blood or liver homogenate incubations. The disappearance of PGMEA was faster in rat blood than in human blood. The hydrolysis half-lives were 36 and 34 min for the 5 and 50 µg/ml concentrations of PGMEA in human blood, whereas in rat blood the hydrolysis half-lives were 16 and 15 min, respectively. The rate of loss of PGMEA was similar in rat and human liver homogenate incubations. Hydrolysis half-lives were 27 and 30 min for the 5 and 50 µg/ml concentrations of PGMEA in human liver homogenate, whereas in rat liver the hydrolysis half-lives were 34 min for both concentrations. These data indicate that PGMEA is rapidly hydrolyzed in the body to its parent glycol ether, PGME. Once hydrolyzed the kinetics for PGME derived from PGMEA is identical to that for PGME. *Sponsored by the American Chemistry Council Glycol Ethers Panel.*

663 DISPOSITION AND ORAL BIOAVAILABILITY OF HALOACETATE MIXTURES IN F344 RATS.

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Chloro, bromo and mixed bromo- chloro haloacetates (HAs) are by-products of drinking water disinfection and exhibit both carcinogenic and reproductive/developmental toxic properties in rodents. Drinking water typically contains a mixture of di- and tri-HAs. Recent toxicokinetic studies have identified significant differences in the disposition of di- and tri-HAs. It is now established that di-HAs are almost exclusively eliminated *via* metabolism that occurs as a glutathione dependent process mediated through a novel class of GST isoenzyme, GST-zeta. Di-HAs are prone to extensive first pass metabolism by the liver that can greatly reduce their oral bioavailability. The brominated tri-HAs are metabolized in the microsomal fraction of rodent and human liver tissue and are also subject to first pass liver metabolism. Recent experiments have shown that metabolism of di-HAs is greatly reduced after prolonged exposures due to suicide inactivation of the GST-zeta protein. Preliminary evidence suggests that the tri-HAs do not exert this effect. We measured the tissue distribution, in-vivo plasma/tissue partitioning and oral bioavailability of two separate HA mixtures containing both di- and tri-HAs in F344 rats before and after depleting GST-zeta activity. Results indicated the plasma/tissue partition coefficients ranged between 0.6-1. The oral bioavailability of tri-HAs was not affected by GST-zeta depletion and ranged between 67-92 %. The oral bioavailability could not be assessed for the di-HAs as the plasma area-under-the-curve was always greater after oral dosing compared to the i.v. reference dose. This phenomenon appears to be due to secondary peaks that appear in the plasma profiles of di-HAs after oral dosing. Supported by EPA STAR grant R825954.

664 STEREOSPECIFIC DISPOSITION OF BROMOCHLORO- AND CHLOROFLUOROACETATE: EFFECT OF GST-ZETA DEPLETION.

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The chloro- and bromo- haloacetates are drinking water disinfection by-products and rodent carcinogens. Chloro, bromo di-haloacetates are also mechanism based inhibitors of glutathione-S- transferase zeta (GSTZ1-1). We studied the stereospe-

cific toxicokinetics of two chiral di-haloacetates in male F344 rats: (-), (+)bromochloro-acetate (BCA), and racemic chlorofluoro-acetate (CFA), a non GST-zeta inhibiting di-haloacetate. These experiments were repeated in animals that had previously been treated with dichloroacetate (DCA) to deplete GST-zeta activity. Results indicated that in naïve rats the elimination half-life of (-)BCA was 0.07 hrs compared to 0.40 hrs for (+)BCA. A comparable difference in elimination half-life was also observed for the CFA stereoisomers (0.79 hr *vs.* 0.11 hrs). In GST-zeta depleted rats, stereospecific elimination of (-), (+) BCA was absent with both stereoisomers having an elimination half-life of approximately 0.4 hrs. This finding was in contrast to results for CFA, which still maintained the same relative difference in elimination rate between its stereoisomers although overall elimination was diminished in GST-zeta depleted rats. The AUC(0-inf) of (-), (+) BCA after oral administration differed by more than 10 fold in naïve rats but this difference was reduced considerably in GST-zeta depleted rats. The oral bioavailability varied between 20 - 67% for the different BCA stereoisomers. These results indicate (+)BCA is a poor substrate for GST-zeta and the internal dosimetry of (-), (+)-BCA will differ considerably after drinking water exposure. Supported by EPA STAR grant R825954.

665 TOXICOKINETICS OF HEXACHLOROBENZENE (HCB) IN FEMALE RATS.

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The toxicokinetics (TK) of hexachlorobenzene were assessed after p.o. (300 µg/kg) and i.v. dosing (100 or 1000 µg/kg) to female Sprague-Dawley rats. TK parameters estimated for the p.o. group included maximum blood levels (C_{max}) and time to peak (T_{max}), elimination rate (k_e) and half-life ($t_{1/2}$), volume of distribution (V_c/F), clearance (Cl/F), dose-normalized areas under the concentration-time curve (DNAUC), and bioavailability (F). Parameters estimated for the i.v. groups were C_{max}, distribution and elimination rates (α and β) and $t_{1/2}$'s, volumes of distribution (V_c and V_{ss}), clearance (Cl), and DNAUC. Liver, fat, and lung were also analyzed for HCB. Kinetic parameters estimated included mean residence time, C_{max}, T_{max}, $t_{1/2}$, $AUC_{0\text{-}inf}$, and DNAUC. Blood levels of HCB showed linear kinetics at the i.v. doses used, and HCB given orally was completely and rapidly absorbed (F = 1.2; T_{max} = 3 hr). TK parameters estimated for the p.o. and i.v. groups were comparable except for Cl. The fact that the data collection period covered only 3 to 4 elimination half-lives, and the final portions of the blood concentration-time curves were flat may explain this. TK parameters for HCB in fat and liver behaved in a similar manner, and suggest linear kinetics over the dose range. HCB was highly concentrated in fat. TK parameters estimated for HCB levels in lung suggest non-linear kinetics. TK parameters for the oral group are consistent with those from an earlier study, where dose normalized peak blood levels (C_{max}) after a p.o. dose at 30, 000 µg/kg were of similar magnitude to those at 300 µg/kg. In the earlier study, T_{max} was at 3 hr, thus indicating linear (first-order) absorption following an oral dose of 300 to 30, 000 µg/kg of HCB. Other parameters, such as Cl/F, F, and the $t_{1/2}$'s were also similar in magnitude. Bioavailability was found to be complete (F = 1.1) for female rats receiving an oral dose of 30, 000 µg/kg, estimated using the average DNAUC from rats in these low and high i.v. dose groups.

666 DISPOSITION OF MENTHOFURAN IN F344 RATS.

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Menthofuran is a proximate toxic metabolite of pulegone, a monoterpene constituent of pennyroyal oil, and is believed to account for a significant percentage of pulegone toxicity. Metabolism of menthofuran yields a highly reactive γ-ketoenal intermediate that covalently binds to protein. A study comparing the disposition of menthofuran to pulegone in F344 rats has been conducted as part of the National Toxicology Program initiative in herbal medicines and dietary supplements. Adult male and female F344 rats received single oral doses of ^{14}C-menthofuran (6 or 60 mg/kg) in corn oil; urine and feces were collected and the animals were sacrificed at 24 or 72 hours for tissue collection. Although menthofuran is fairly volatile, only 0.22% of the original dose was recovered as expired volatiles. Overall, 92 to 98% of the dose was recovered. Menthofuran is excreted in the urine (57 to 65% after 72 hours) and feces (19 to 28%). The highest concentrations of menthofuran-derived radioactivity were in blood and liver at 24 and 72 hours. The concentration of radioactivity in kidneys from males was 2-fold higher than those from females at 24 hours. The sex-related difference in kidney concentration is not as great as observed for pulegone, but the results may indicate that menthofuran and/or a metabolite(s) binds to α2u globulin. At 72 hours, 10 to 14% of the dose was still in the tissues. This is 3-times the residual pulegone in tissues at 72 hours at a similar dose. Menthofuran-derived radioactivity was covalently bound to liver tissue with 0.98 and 0.91 nmole-eq/mg protein in male rats dosed with 6 mg/kg menthofuran for 24 and 72 hours respectively. The extent of covalent binding to liver protein is high and is comparable to furan. The observation of higher residual radioactivity in tissues compared to pulegone and covalent binding to protein is consistent with menthofuran being a proximate toxic metabolite of pulegone.

667 THE DOSIMETRY AND MODES OF ACTION OF 2-BUTOXYETHANOL IN B6C3F1 MICE.

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2-Butoxyethanol (BE) is a widely used solvent in industrial and consumer cleaning and coating products. An extensive toxicity and mechanistic database shows humans are considerably less sensitive than rats and mice to hemolysis, the main endpoint of toxicity. However, a recent NTP inhalation bioassay identified the forestomach and liver of mice, but not rats, as target organs for potential tumorigenicity. A series of studies were conducted to evaluate the role of dosimetry in producing lesions specifically in the forestomach. Results to date indicate that forestomach tumors appear to be produced by a chronic irritation/cytoxicity mechanism. This further questions the relevance of the forestomach tumors for humans which lack this tissue. Irritation and compensatory hyperplasia were induced in the forestomachs of mice upon oral administration of neat BE. Similar, but milder lesions were also produced by systemic administration (intraperitoneal or subcutaneous injection) of comparable doses. Salivary excretion was found to be a contributing source of BE and its metabolite, butoxyacetic acid (BAA) to forestomach tissue in female mice following intraperitoneal injection. Salivary levels of BE in the mice were nearly equivalent to blood concentrations. The elimination half-lives for BE and BAA in blood were 0.06 and 0.9 hr, respectively, which is considerably faster than rats or humans. Based upon focused kinetic and toxicity studies following inhalation, oral and intraperitoneal injection routes of exposure, sources of BE and its hemolytic metabolite, butoxyacetic acid (BAA) in the forestomachs of mice can be attributed to a combination of systemic delivery *via* the blood, elimination in saliva with subsequent oral ingestion and oral consumption due to grooming of BE deposited on the fur. Taken together, there appears to be a significant role for local tissue dosimetry in the development of forestomach cytotoxicity in mice. (Sponsored by the Ethylene Glycol Ethers Panel of the American Chemistry Council).

668 DISPOSITION OF POLYDIMETHYLSILOXANE, 10 AND 350 CST IN F344 RATS FOLLOWING A SINGLE EXPOSURE BY ORAL GAVAGE.

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Disposition of two neat ^{14}C-polydimethylsiloxanes (PDMS) with viscosities of 10 and 350 cst from the GI tract was evaluated in male and female Fischer 344 rats. A single, oral dose of 1000mg/kg of body weight was administered to rats in Mass Balance (MB: 4 females and 4 males) and Whole Body Autoradiography groups (WBA: 4 females and 4 males). Rats in the MB groups were housed in Roth-style metabolism cages for collection of excreta and the assessment of the recovery of total radioactivity. At 96 hr post-dose, rats were euthanized by cardiac puncture under anesthesia and blood, liver, GI tract, kidneys, spleen, eyes, perirenal fat, and remaining carcasses were collected and processed for analysis. Radioactivity content in each sample was measured by liquid scintillation counting (LSC). Aliquots of the fecal homogenates were extracted and analyzed by HPLC with radiochemical detection using a gel-permeation chromatography column for qualitative determination of the radioactivity profile. Rats in the WBA groups were sacrificed at 12, 24, 48 and 96 hr post-dose and immediately frozen at -75°C. Sagittal sections of 40 µm in thickness were collected at various levels to include major organs and tissues and exposed to x-ray film at -80°C. Both quantitative (LSC) and qualitative assessments (WBA) showed that radioactivity was rapidly excreted through gastrointestinal tract. *in vivo* assessment of tissue distribution by WBA at 12, 24 and 48 hr post-dose showed that radioactivity was concentrated in the contents of the GI tract. No significant radioactivity was detected outside the GI tract with the exception of exterior contamination. Dose recovery from all the animals through 96 hr post-dose was > 93.6%. Virtually the entire recovered dose was excreted in feces (99.6-99.8% of recovered dose) with little, if any, absorption. The entire radioactivity recovered in feces was attributed to the unchanged PDMS, 10 or 350 cst. No detectable degradation products were present in feces. Funded in part by Silicones Environmental, Health and Safety Council.

669 CHROMIUM ACCUMULATION IN THE TISSUES OF RATS, MICE, AND GUINEA PIGS EXPOSED TO SODIUM DICHROMATE DIHYDRATE IN DRINKING WATER FOR TWENTY-ONE DAYS.

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Chromium (Cr) is widely used in industry for corrosion inhibition, metal finishing, manufacturing of safety matches and production of pigments. Hexavalent chromium (VI) has been found to contaminate soil and air. This study was designed to evaluate the relationship between drinking water concentrations of Cr (VI), as sodium dichromate dihydrate (SDD), and tissue accumulation of total Cr in three species. Male Fischer 344 rats, B6C3F1 mice, and Hartley guinea pigs were exposed to SDD in tap water for 21 days at 0, 1, 3, 10, 30, 100, or 300 mg Cr/L water (0, 2.87, 8.62, 28.7, 86.2, 287, and 862 mg SDD/L; n=4/dose). On Study Day 22 until necropsy, all animals received tap water (0 mg Cr/L). Actual doses received were calculated based on twice weekly water consumption data and weekly body weights. On Study Day 24, animals were humanely sacrificed and blood, kidneys, and femurs were collected. Whole blood and tissues were analyzed for total chromium content. Water consumption remained normal throughout the course of the study for rats and mice and did not differ between dose groups (water consumption could not be calculated for guinea pigs). In mice, mean body weights were not significantly different from controls. However, for both rats and guinea pigs, mean body weights at the 100 and 300 mg/L dose levels were decreased when compared to controls. Cr levels in rat kidney were ca. 3 times higher than in mouse kidney (rat range: 0.4 – 9.1 µg/g *vs.* mouse range: 0.1 – 3.3 µg/g). Cr levels in guinea pig kidney were similar to mouse kidney (range: 0.1 – 3.1 µg/g). In all three species, Cr tissue concentrations appear to increase in a linear manner up to 80 mg Cr/L. Tissue concentrations of Cr do not appear to increase at higher dose levels. This may be due to a decrease in the bioavailability of Cr or a decrease in the uptake of Cr into specific tissues. Tissues appear to reach a saturation level at the 100 mg Cr/L dose level.

670 BIOAVAILABILITY OF PENTACHLOROPHENOL FROM DIFFERENT TYPES OF SOILS.

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Pentachlorophenol (PCP) is a broad-spectrum biocide widely used as a wood preservative and is thus a ubiquitous environmental pollutant. Evidence exists that usually only a fraction of the chemicals in soil is bioavailable to organisms, and this bioavailability is influenced by chemical and soil properties. In the current study, the absolute and relative bioavailabilities of PCP from soils varying in organic carbon content, particle size and pH were examined. Four uncontaminated soils and two contaminated soils from a wood preserving industry were collected from different sites in Indiana. Uncontaminated soils were spiked with 50 and 100 mg/kg PCP. The PCP concentration in the soils from contaminated sites was approximately 200 mg/kg. Soils and corn oil containing equivalent levels of PCP were administered to male Sprague-Dawley rats (weight range 275 to 350 g) by gavage at two dose levels—100 and 200 µg/kg body weight. Equivalent doses were also given *via* intravenous injection (iv). Blood samples were obtained from the orbital sinus at 1, 3, 6, 12, 24, and 48 hours after administration. PCP concentrations in plasma were analyzed by GC-ECD. Areas under the curve (AUC) were calculated for each soil and compared to those of the iv injection and corn oil groups to estimate the absolute bioavailability (AUC of soil:AUC of iv injection) and relative bioavailability (AUC of soil:AUC of corn oil). Both absolute and relative bioavailabilities were very high for each soil at each dose with no statistical differences detected among them. Bioavailabilities decreased in one of the environmentally contaminated soils. These results indicate that PCP in soil is readily absorbed into the blood from the gastrointestinal tract and the soil properties do not have much impact on PCP bioavailability. (Supported by EPA grant CR827234).

671 TIME COURSE FOR TISSUE DISPOSITION OF DIMETHYLARSINIC ACID AFTER ACUTE ORAL ADMINISTRATION IN THE MOUSE.

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The primary metabolite excreted by most mammals after exposure to inorganic arsenic is dimethylarsinic acid (DMA(V)). Recent studies have shown that DMA(V) is a multi-organ tumor promoter in rodents and a bladder carcinogen in rats. This study examined the disposition of DMA-derived radioactivity in tissues over time after acute oral administration. Adult female B6C3F1 mice (N=3-4/time point) were administered [^{14}C]-DMA(V) orally at a dose level of 0.6 or 60 mg As/kg, and sacrificed at selected time points (0.25-24 hr). Tissues were removed and analyzed for chemical-derived radioactivity (^{14}C). DMA(V) was rapidly absorbed and distributed among the tissues, as evidenced by the detection of ^{14}C in all tissues at the earliest time point (0.25 hr). Concentrations of ^{14}C (%dose/ gm tissue) in the kidney, liver, and lung were greater than in blood at this time, indicating that DMA is rapidly accumulated by tissues. The peak concentration of ^{14}C in the tissues occurred between 0.5-2 hr after administration of DMA(V) and ranged from 0.4-2.6%. The tissues with the highest concentration (2.6%) of ^{14}C were the lung for the low dose and the kidney for the high dose. There were no dose-dependent differences in peak tissue concentration of ^{14}C. By 24 hr, the tissue concentration of ^{14}C was considerably lower than observed at 0.25 hr. Approximately 0.01% of the dose/gm tissue or lower was detected in kidney, liver, or lung at 24 hr. The concentration of ^{14}C in these tissues was greater than in the blood at 24 hr. The data indicate that an acute oral dose of DMA(V) is rapidly absorbed, distributed, and excreted from the mouse. The rapid elimination of DMA(V) explains in part the low acute toxicity of this compound. (This abstract does not necessarily reflect EPA policy.)

672 THE POTENTIAL OF SELECTED CROP PROTECTION CHEMICALS TO ALTER HUMAN P-GLYCOPROTEIN ATPASE ACTIVITY.

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P-glycoprotein (PGP, encoded by mdr1) is a member of the ABC transporter superfamily and is expressed in the capillary endothelial cells of the brain, gonads, placenta and other tissues. PGP serves as a barrier to keep toxins out of the nervous system, gonads and fetus by actively pumping substrates across the cell membrane and out of the cytoplasm; ATP hydrolysis provides the energy for active transport. Numerous drugs and crop protection chemicals (CPC) are substrates for PGP [Bain and LeBlanc, 1996, *Toxicol. Appl. Pharmacology* 141, 288-298]. The potential of selected CPC to stimulate the ATPase activity of human PGP membranes(GENTEST™ Catalog K228) was evaluated. CPC were incubated in a buffered solution containing PGP membranes and supplemented with MgATP, with and without sodium orthovanadate to inhibit PGP by trapping MgADP in the nucleotide binding site. Each CPC was assayed over a concentration range based on its solubility in DMSO up to 100 µM in 96-well microtiter plates. The herbicides, atrazine and clodinofop-propargyl, did not stimulate human PGP-ATPase activity in the concentration ranges tested nor did the insecticide, thiamethoxam. The failure of these substances to alter ATPase suggests they are not substrates of PGP. The insecticide, emamectin-benzoate, suppressed the basal activity in the PGP membranes over the entire concentration range tested, indicating an inhibitory interaction with the PGP-ATPase. Additionally, when verapamil, an established substrate of PGP was coincubated with increasing concentrations of emamectin-benzoate, the verapamil-stimulated ATPase activity was inhibited in a concentration-dependent manner. The triazine herbicide didealkylated metabolite, diaminochlorotriazine, as well as the fungicide, propiconazole, stimulated activity in a concentration-dependent manner indicating that they are substrates for PGP. This screen further supports the premise that CPC, like pharmaceutical agents, may interact with PGP.

673 DISTRIBUTION OF CHLORPYRIFOS AND ITS PRINCIPAL METABOLITE TRICHLOROPYRIDINOL IN DAM AND NEONATE TISSUES/FLUIDS.

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Pregnant Sprague-Dawley rats were administered chlorpyrifos (CPF) *via* gavage (in corn oil) at doses of 0.5, 3.0, and 5.0 mg/kg from gestational day 14 to postnatal day 7. CPF and its principal metabolite, trichloropyridinol (TCP), were assayed in the samples collected on postnatal day 7 from dams (blood, brain, liver and milk) and neonates (plasma, brains, and livers) at Battelle using gas chromatography-negative-ion chemical ionization mass spectrometry (GC-NCI/MS) methods. CPF was readily quantified in dam milk, liver, brain, and blood collected from dams administered 5 mg CPF/kg, but was not detectable in neonate plasma, liver, or brain from the 5 mg CPF/kg group. CPF was also readily quantified in liver and detectable in brain samples collected from dams administered 3 mg CPF/kg. Generally, CPF concentrations in all study samples were not detectable in samples from either the control or 0.5 mg CPF/kg groups. In those tissues/fluids where CPF was observed, the concentrations increased with increasing CPF dose and the highest CPF concentrations were observed in dam milk. TCP was quantifiable in all tissues/fluids analyzed (blood, liver, brain, milk) from dams administered 0.5, 3.0, and 5.0 mg CPF/kg. TCP was detectable in plasma, liver, and brain collected

from neonates in the 0.5, 3.0, and 5.0 mg CPF/kg groups. TCP concentrations in the dam and neonate tissues/fluids increased with increasing CPF dose. Although TCP was detected in all dam blood samples, a dam liver sample, four neonate plasma samples, and four neonate brain samples that were part of the control group, mean TCP concentrations were below the LOD, or had measurements that were suspect due to contamination. Overall, the highest concentrations of TCP were observed in dam blood and liver. TCP concentrations in neonate plasma and liver were greater than TCP concentrations in neonate brain.

674 KINETICS OF PERCHLORATE-INDUCED INHIBITION OF IODIDE UPTAKE IN TISSUES OF THE PREGNANT RAT AND FETUS.

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Perchlorate (ClO_4^-) acts as a competitive inhibitor for iodide uptake into the thyroid gland. This results in perturbation of biosynthesis of thyroid hormones necessary for neurodevelopment in prenatal and neonatal periods. The objective of this project was to determine disposition of ^{125}I in maternal and fetal tissues of Sprague-Dawley rats, and inhibition of iodide uptake in maternal thyroids. Pregnant rats (GD20, n=6) were intravenously injected with carrier-free ^{125}I (1.87 ng/kg, control) or with 1 mg/kg ClO_4^-, then with carrier-free ^{125}I 2h later. Dams were sacrificed at 0.5, 1, 2, 4, 8, 12 and 24h post dosing. Blood, thyroid, skin, placenta, mammary gland, amniotic fluid, gastric tract (GT) and gastric contents (GC) were collected as well as fetal blood, skin and GT. Percent inhibition of iodide uptake in dam's thyroid was observed at all time points, the highest (over 85%) at 1 and 2 h, and the lowest (over 65%) at 0.5 and 24 h post dosing of iodide. Concentrations of iodide in control serum at 0.5, 1, 2, 4, 8, 12 and 24 h were 2.99, 2.52, 2.35, 1.84, 1.26, 0.36 and 0.014 pg/ml. Iodide levels in control dam's serum and skin were similar during 24h period while iodide concentrations of control fetal serum and skin at 0.5, 1, 2, 4 and 8h were 0.81, 1.08, 1.03, 1.13, 0.57 and 1.10, 2.02, 1.37, 1.36, 1.56 pg/ml, respectively. Levels of iodide in amniotic fluid were lower than fetal skin levels except 24 h. Iodide levels of control dam's mammary gland at 4, 8, 12 and 24h were 2.41, 2.19, 2.38 and 1.49 pg/ml, respectively, and these are higher than dam's serum levels. Sequestration of iodide was evident in both fetal skin and dam's mammary gland. This information was used to develop a physiologically based pharmacokinetic model to illustrate target concentrations of ClO_4^- in prenatal period. In addition, this model will be employed to address potential risk associated with ClO_4^- exposure during gestation.

675 KINETICS OF PERCHLORATE AND IODIDE IN LACTATING S-D RATS AND PUPS AT POSTNATAL DAY 10.

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Ammonium perchlorate (NH_4ClO_4), a groundwater contaminant resulting from military and industrial use, readily dissociates to NH_4^+ and ClO_4^-. The perchlorate ion (ClO_4^-) competes with I^- for uptake into the thyroid, potentially causing hypothyroidism. Time course studies were performed to understand better the kinetics of ClO_4^- and iodide in lactating dams and nursing pups. At postnatal day (PND) 10 female Sprague-Dawley dams were intravenously (i.v.) injected with 0.1 mg/kg ClO_4^- in water and returned to their culled litters. Dam serum, thyroid, skin, mammary gland and gastric contents and pup serum, skin, gastric tract and contents were collected at 0.5, 1, 2, 4, 8 and 12 hr for ClO_4^- analysis. To investigate the inhibition of iodide uptake by ClO_4^-, lactating dams at PND 10 were i.v. injected with 1 mg/kg ClO_4^- followed 2 hr later with an i.v. injection of carrier free ^{125}I and returned to their litters. Control sets of lactating dams were i.v. injected with carrier free ^{125}I only and returned to their litters. Dam serum, thyroid, skin, mammary gland and gastric contents and pup serum, skin, gastric tract and contents were collected at 0.5, 1, 2, 4, 8 and 24 hr for ClO_4^- and iodide analysis. Perchlorate level in serum after dosing with 0.1 mg/kg ClO_4^- was 0.19 ± 0.02 mg/ml at 4hr and was not detectable at 8 hr. Both male and female pups had 0.038 ± .003 mg/ml of ClO_4^- in serum at 12 hr. Perchlorate exposure inhibited iodide uptake into thyroids of lactating dams. At 30 min the bound iodide level in dam thyroid after ClO_4^- exposure was 0.19 ± .02 ng/g whereas the level in dam thyroid after iodide only exposure was 0.08 ± .03 ng/g. The information generated from this kinetic study will support the development and validation of a ClO_4^- lactation model. A risk assessment for ClO_4^-, supported by a lactation model, will address the risks associated with ClO_4^- exposure in a sensitive population.

676 ETHYLENE GLYCOL KINETICS IN PREGNANT RATS: DIFFERENCES BETWEEN SLOW AND FAST DOSE-RATE EXPOSURES.

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Large bolus doses of ethylene glycol (EG) are developmentally toxic in rodents. However, human occupational and consumer exposures typically involve low doses and slow dose-rates (e.g. dermal, inhalation). This study examined the impact of dose-rate on maternal and embryonic kinetics of EG and its metabolites. Pregnant CD rats were dosed from gestation days (GD) 6-16 with 1000 or 2000 mg/kg/day of EG given either as a daily subcutaneous (SC) bolus injection (fast dose-rate) or constant rate infusion (slow dose-rate) *via* an implanted pump. Rats were also dosed by oral gavage (fast dose-rate) on GD 11 at either 100 or 1000 mg/kg. EG and its developmentally toxic metabolite, glycolic acid (GA) were determined in maternal blood samples on GD 7-15 in the SC bolus injection and continuous infusion study. Detailed analysis of the kinetics of EG, GA and oxalic acid (OX) were also determined in maternal blood, urine and kidneys, as well as conceptus fluids and embryos on GD 11-12 for the oral gavage and SC infusion studies. EG levels were similar in maternal blood *vs.* maternal tissues and conceptuses within a given treatment group, while GA levels were consistently higher in tissues than maternal blood. OX levels at all dose levels were variable but similar to control levels in all samples regardless of dose. Blood levels of EG and GA were similar following the two bolus administration routes. However, peak blood levels for both EG and GA were consistently higher (~5-fold for EG and ~50-fold for GA) following bolus oral and SC dosing than following constant rate SC infusion at comparable total dose levels. The lower levels of GA observed in the slow dose-rate group corresponded with the low toxicity characteristic of slow dose-rate administration of EG, as shown in previous studies. Therefore, bolus dosing with EG appears to significantly overestimate the risk of typical occupational and consumer exposures to EG. Funded by the American Chemistry Council, Arlington, VA.

677 BISPHENOL A (BPA): THE EFFECT OF AGE AND DOSE ON THE *IN VIVO* METABOLISM AND PHARMACOKINETICS FOLLOWING ORAL ADMINISTRATION TO MALE AND FEMALE NEONATAL AND ADULT SPRAGUE-DAWLEY RATS.

J. Y. Domoradzki[1], S. C. Hansen[1], C. M. Thornton[1], T. L. Card[1], M. D. Dryzga[1], J. M. Waechter[1] and R. N. Shiotsuka[2]. *[1]The Dow Chemical Company, Midland, MI and [2]Bayer Corp., Stillwell, KS.*

A previous study demonstrated the rapid clearance of BPA from blood following oral administration to adult rats with the principal metabolite being BPA-monoglucuronide (Toxicology Sciences 54: 3-18, 2000). Since the ontogeny of certain isozymes of glucuronyl transferases (GT) differ with age, the pharmacokinetics of BPA was studied in neonatal animals. ^{14}C-BPA was administered *via* oral gavage at 1 or 10 mg /kg bw in corn oil, to rats at post-natal day (pnd) 4, pnd 7, pnd 21, and 11-week old adults. Blood was collected at 0.25, 0.75, 1.5, 3, 6, 12, 18 and 24-h post-dosing. BPA and BPA-glucuronide in plasma were quantified by HPLC with eluent fractions collected for liquid scintillation spectrometry analysis. The data indicate that neonates at all three ages metabolized BPA to its monoglucuronide. The majority of the plasma radioactivity following administration of the higher dose was BPA-monoglucuronide with concentrations about 100-fold greater than the plasma concentrations of BPA, except at the earliest times post-dosing. BPA-monoglucuronide and BPA concentrations in plasma of neonates were greater than in adults, except at 24 h. BPA was not detectable in adult female plasma, except at 0.75-h post-dosing, and by 3-h in males. At a dose of 1 mg/kg, plasma radioactivity concentrations were ~10-fold lower than at a dose of 10 mg/kg and BPA-monoglucuronide was the only metabolite observed in plasma representing 91-100% of the radioactivity present. A clear dose dependency in the metabolism and pharmacokinetics of BPA administered to neonates was observed with nearly complete metabolism of BPA to BPA-monoglucuronide at a dose of 1 mg/kg. These data suggest GTs that can metabolize BPA to its monoglucuronide develop sufficiently early in rodents to efficiently metabolize low doses of BPA to its non-estrogenic metabolite. Sponsored by: PC/BPA Global Group, American Plastics Council, Arlington, VA.

678 METABOLISM AND PHARMACOKINETICS OF BISPHENOL A FOLLOWING ORAL ADMINISTRATION TO PREGNANT SPRAGUE-DAWLEY RATS.

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A previous study demonstrated the rapid clearance of BPA from blood following oral administration to rats (Toxicology Sciences 54: 3-18, 2000). However, the pharmacokinetics of BPA has not been extensively studied in pregnant animals. In

this study, ^{14}C-BPA was administered *via* gavage at 10 mg BPA/kg body weight to time-mated pregnant rats on gestation days 6, 14, 17, and to non-pregnant female rats. Blood samples were collected at selected time points over a 96-h period following oral administration. Plasma radioactivity, BPA and BPA-glucuronide were quantified in pooled plasma samples by liquid scintillation counting or radiochemical profiling using HPLC. Pooled excreta samples from selected dose groups and collection intervals were profiled by HPLC to quantify total BPA parent, BPA-glucuronide and metabolites. Structural confirmation of BPA and BPA-glucuronide were confirmed, as in a previous study, for representative plasma, urine and fecal samples. Selected tissues were collected at sacrifice and analyzed for radioactivity, parent compound and metabolites, including blood, brain, embryo/fetus, fat, liver, kidneys, ovaries, placenta, uterus, skin, and remaining carcass. BPA pharmacokinetics and metabolism did not appear to be affected by pregnancy. There was evidence of enterohepatic circulation in all groups, based on a secondary peak in the plasma radioactivity concentration-time profile. Mean tissue concentrations were either non-quantifiable or <0.25 µg-equivalents/g tissue. No apparent selective affinity of either placenta or embryo/fetus for ^{14}C-BPA-derived radioactivity was observed. Systemic exposure to parent was very low based on plasma BPA concentrations. BPA-monoglucuronide was found to be the major metabolite in both pregnant and non-pregnant rats. This study provides important evidence that the disposition of BPA is not altered by reproductive status. *Sponsored by: PC/BPA Global Group, American Plastics Council, Arlington, VA.*

679 PHARMACOKINETICS OF ^{14}C-GENISTEIN FOLLOWING I.V. ADMINISTRATION IN ADULT FEMALE SPRAGUE-DAWLEY RATS.

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Genistein is a phytoestrogen present in soy-food and virtually all rodent diets and has been reported to elicit detectable estrogenic effects in developing rodents. Understanding the placental transfer of genistein in rats as a possible route of exposure during development will be important for assessing potential effects. As a first step this study was designed to evaluate the clearance of genistein from blood of nonpregnant female Sprague-Dawley rats dosed with ^{14}C-genistein. The time profile of genistein blood kinetics was established following intravenous administration of a high dose (40 mg/kg b.w.) and low dose (4 mg/kg b.w.) of ^{14}C-genistein (10 µCi/rat; 1 ml/kg, in 65% DMSO) 48 hours following jugular vein cannulation. Blood samples were taken at 0, 5, 15, 30, 45, 60, 90, 120, 180, and 240 minutes following dosing and total radioactivity in plasma determined by liquid scintillation spectrometry. Five minutes after the administration of genistein, the plasma concentration was 130 ± 28 and 7.9 ± 2.7 µg genistein equivalents/ml plasma following high and low dose, respectively. These plasma levels rapidly declined so that by one hour the levels were 16 ± 7.1 and 0.9 ± 0.2 µg genistein equivalents/ml in the high and low-dosed animals, respectively. Fitting the plasma concentration-time curves to a 2-compartment model the initial half-life (alpha-phase) was estimated to be ~ 12 min for the 40 mg/kg b.w. dose and ~ 7 min for the 4 mg/kg b.w. dose. The terminal half-life (beta-phase) derived from the curve of the high dose was approximately 2.3 hr. Because the levels of genistein fluctuated during the terminal phase of elimination following the low dose, this half-life could not be determined. These fluctuations may be influenced by enterohepatic circulation of genistein which is known to occur in rats. These data provide the kinetic information that can be used in conjunction with a PBPK model to estimate metabolic rate constants for genistein. This study was funded in part by the American Chemistry Council.

680 DISTRIBUTION OF MANGANESE IN THE BRAIN OF STAINLESS STEEL WELDING FUME INHALED SPRAGUE-DAWLEY RATS.

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Welders working in the confined space like shipbuilding industry have a risk of exposing a high concentration of welding fumes and of developing pneumoconiosis or other welding fume exposure related diseases. Among them, manganism caused by welding fume exposure is still controversial and no clear demonstration of manganese movement into specific brain regions. To investigate distribution of manganese in the brain of welding fume exposed rats, welding fumes generated from manual metal arc stainless steel (MMA-SS) were exposed to male Sprague-Dawley rats with concentrations of 63.6 ± 4.1 mg/m^3 (low dose) and 107.1 ± 6.3 mg/m^3 (high dose) total suspended particulates for 2 hrs per day, in an inhalation chamber for 90 days. Blood, brain, lung and liver were collected at the end of 1. 15, 30, 60, and 90 days of exposure, and the tissues were analyzed for their manganese concentrations by atomic absorption spectrophotometer. Although there were dose dependent increases of manganese concentrations in the lungs of exposed rats during 90 days of exposure, only slight manganese increases were detected in the blood during exposure period. Major statistically significant increases of manganese concentrations in the brain were detected in the cerebellum after 15-60 days of exposure. There were slight increases of manganese concentrations in the substantia nigra, basal ganglia (caudate nucleus, putamen and globus pallidus), temporal lobe and frontal lobe.

681 CELL TYPE SPECIFIC DIFFERENCES IN FORMATE TOXICITY.

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Methanol is an important health and environmental concern because of the neurotoxic actions of its metabolite, formic acid. Formic acid has been hypothesized to act as a mitochondrial toxin to produce retinal and optic nerve toxicity. Studies in our laboratory using a methanol-sensitive rodent model have revealed functional, metabolic and structural abnormalities in the retina consistent with this hypothesis. The present studies were undertaken to further define the mechanism of formate toxicity by examining cell-type specific differences and cellular consequences of formate toxicity. Madin-Darby canine kidney cells (MDCK), retinal pigment epithelial cells (RPE), and a photoreceptor cell line (661W) were exposed to formate (1-100mM) over 3 time courses (10 min, 24 hrs, and 13 dys). Cellular formate concentrations, morphologic changes, and cellular ATP concentrations were assessed. Intracellular formate concentrations increased to a similar extent in all 3 cell types. In cultures exposed to 10 mM formate, concentrations increased from basal concentrations of 8.8 ± 1.1 µmoles formate/mg protein to a plateau concentration of 115.2 ± 12.5 µmoles formate/mg protein following 6 dys of exposure. In cultures exposed to 100mM formate, intracellular formate concentrations increased for 6 dys plateauing at 253.4 ± 21.4 µmoles formate/mg protein. Formate treatment produced morphologic changes in MDCK cells primarily characterized by cell swelling. No morphologic alterations were observed in RPE or 661W cells. Intracellular ATP concentrations were significantly decreased in MDCK cells and RPE cells within 24 hours. In 661W cells, ATP concentrations increased from 15 ± 2 to 26 ± 3 nmoles ATP/mg protein within minutes followed by a significant decrease in ATP concentrations to 5 ± 1 nmoles ATP/mg protein by 6 hrs. These data provide evidence of cell-type specific differences in the cytotoxic actions of formate. They further suggest that intracellular compensatory mechanisms are rapidly triggered following formate exposure. (RO1-ES06648, RO1-EY11396 and P30-EY01931).

682 ESTIMATING MINIMUM CRITERIA FOR JUDGING THE PERFORMANCE OF TOXICITY TESTS IN VALIDATION STUDIES.

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It is often stated that a new toxicity test (NTT) must provide a level of protection equivalent to or better than the reference test it will replace. It is less clear how to measure the performance of a reference test in a manner that is useful for comparison with a NTT. The purpose of this report is to describe an approach used to define the predictive capacity of a commonly used *in vivo* method, the Draize eye irritation test. Data were obtained on the reproducibility of the Draize test from the scientific literature. These data were then used to define variability terms used in a computer simulation that models the predictive capacity of the Draize test. The validity of the simulations was verified by comparing actual *in vivo* data with results from the simulations. The Draize test variability is greatest for the mildest irritants. The maximum expected correlation coefficient is r = 0.84. The expected sensitivity = 0.74-0.95, specificity = 0.67-0.87, and concordance = 0.82-0.92 depending on the cutoff chosen. The width of the 95% prediction interval ranges between 16 and 93 for a predicted maximum average score of 55 on the 110-point scale. These performance statistics can be used as baseline criteria for judging the adequacy of NTT intended to replace the *in vivo* test.

683 ESTIMATION OF DNA STRAND BREAKS IN LENS EPITHELIAL CELLS: WHITE LIGHT MEDIATED GENOTOXICITY.

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It was hypothesized that white light induces DNA single-strand breaks/alkali labile sites in lens epithelial cells of 60 day old Brown Norway rats during the dissection process. Rats were narcotized using carbon dioxide gas and eye lenses were dissected

out either in white or red light at a distance of 3.5 inches and for a duration of approximately five minutes. Electrical input to lamps was adjusted such that the light delivered from each was approximately equal to the intensity of 100 foot candles (1076 lux). DNA single strand breaks/alkali labile sites were estimated using the alkaline microgel electrophoresis (COMET) assay. Dissection of lenses in white light induced significantly higher integrated intensity ($p=0.0216$), tail moment ($p=0.0453$), comet extent ($p= 0.0462$, tail extent ($p=0.0048$) and tail breaks ($p=0.048$) as indices of DNA single strand breaks/alkali labile sites. In addition, we investigated the DNA damaging effect of visible light in a human lymphocyte model in which cells were exposed to white, red or minimal indirect light for five minutes. Results of these experiments confirm our conclusion regarding the ability of visible light to induce DNA damage in individual, isolated cells. Our results on the ability of visible light to induce DNA single strand breaks/alkali labile sites may have numerous implications in both plant and animal cells. These findings may also relevant while conducting genotoxicity assays on cells *in vitro*.

684 VALIDATION OF A MODEL OF PHOTORECEPTOR DEGENERATION IN THE RAT.

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A rat model of light-induced photoreceptor degeneration was validated in order to serve as an efficacy model for therapeutics directed towards prevention of retinal damage. The design included a positive control group which received an intravitreal injection of brain-derived neurotrophic factor (BDNF) in one eye and the negative control (saline) in the contralateral eye. A negative control group received the intravitreal injection of saline in one eye, while the contralateral eye served as an untreated control. Animals were housed under reduced illumination (£20 foot candles) and 12 hour light cycle, for 10 days prior to injection. Twenty-four hours following the injections, the rats were placed into housing units which ensured continuous and complete (even distribution) light exposure at approximately 200 foot candles, for 84 hours. Thereafter the animals were returned to standard caging, with a 12 hour light cycle, where they were maintained for 14 days. A series of scotopic and photopic electroretinograms (ERGs) were performed prior to the injections and again at the end of the 14 day observation period. Following termination, 5 serial sections of each eye (from inferior to superior eye, including the outer nuclear layer) were prepared and examined histopathologically. Both ERG and histopathology examinations confirmed the light exposure to have induced photoreceptor degeneration in negative control and uninjected eyes. Anticipated neuroprotective action of the positive control compared to the negative control was also evident and therefore the model was considered suitable for use in efficacy evaluations of neuroprotective agents in retinal indications.

685 ROLE OF LENTICULAR CHOLESTEROL BIOSYNTHESIS AND SMALL GTP-BINDING PROTEINS IN THE CJ-12, 918 RAT CATARACT.

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Purpose: Alterations in cholesterol biosynthesis, either through drug inhibition (i.e. lovastatin and triparanol) or inherited genetic defects, are associated with cataract formation in animals and humans. We have previously reported CJ-12, 918 inhibits lenticular cholesterol biosynthesis before significant alterations in lenticular ATP, GSH, and γ-crystallin content, or clinical evidence of cataract formation in the rat. Rao *et al.* have demonstrated *in vitro* that lovastatin inhibits the isoprenylation of small GTP binding proteins in lens epithelial cells (LEC) and have proposed this as the main mechanism of the lovastatin *in vitro* rat and *in vivo* canine cataract. The following study investigates whether CJ-12, 918, like lovastatin, alters small GTP binding protein isoprenylation in primary LEC. Methods: Rat and canine LEC were treated with CJ-12, 918 (10-50 μM) or lovastatin (10-50 μM) for 48 hrs. RhoA/RhoB and Ras isoprenylation expression were analyzed in the membrane/cytosol fractions, and phosphorylated ERKII in the whole cell lysate by Western blot. Results: No change in rat or canine LEC isoprenylation patterns or phosphorylated ERKII was observed with CJ-12, 918 treatment. However, treatment with lovastatin decreased Ras and Rho expression in the membrane fraction with a concurrent increase in the cytosol fraction and decreased phosphorylated ERKII in the whole cell lysate. In the canine LEC, these isoprenylation changes with lovastatin treatment were shown to be concentration-dependent. The effects of lovastatin treatment (50 μM) in the rat LEC were alleviated with mevalonic acid supplementation (100 μM). Conclusions: Our data support lovastatin inhibition of small GTP binding protein isoprenylation in LEC *in vitro*. CJ-12, 918 does not inhibit small GTP binding protein isoprenylation. The major mechanism of the CJ-12, 918 cataract appears to be inhibition of lenticular cholesterol biosynthesis late in the pathway.

686 OCULAR IRRITANCY OF COMMON CONSUMER PRODUCTS USING THE CULTURED BOVINE LENS.

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In recent years, abattoir-supplied bovine lenses have been cultured to measure ocular irritancy as an alternative to the Draize Test. This approach uses cultured cells and a laser-scanning instrument to measure the optical quality of bovine lenses exposed to potentially harmful agents. Quantitative results are obtained rapidly and objectively, and with greater sensitivity, than the Draize Test or other suggested alternatives such as BCOP. We report here the results of a study, which measures the ocular irritancy caused by common semi-solid and solid materials that are partially or not transparent. The consumer products tested included 2 shampoos, 4 body washes, 2 lotions, 3 toothpastes, 3 facewashes, as well as, a deodorant and an antiperspirant; for a total of 16 products. The number of lenses tested per product ranged from 7 to 11, with a total of 153 tested lenses. An additional 28 untreated lenses were tested as controls. Each treated lens was exposed to a test material for 15 minutes at room temperature. The material was then washed off the lens with sterile salt solution and culture medium and the lens was then cultured, as before the exposure, at 37 degrees Celsius. Lens optical function was measured periodically for eight days. Lens damage, as indicated by increased focal variability (loss of sharp focus), appears between 1.7 and 123 hours after exposure. Thus, the results indicate large ranges in ocular irritability on the basis of time to induce damage. This wide range is true both between groups of products and within each group. The deodorant /anti-perspirant results indicate most damage (about 2 to 4 hours) while, on average, the shampoos and the then the body washes are least damaging (about 90 hours and 21 hours, respectively). This study supports an earlier preliminary one showing that the cultured bovine lens can be used to measure ocular irritancy of semi-solid and non-transparent substances. It also demonstrates the sensitivity needed to produce a rank order of irritancy within the various products groups.

687 INTERIM DATA FOR A NINE-MONTH LOCAL TOLERANCE AND TOXICITY STUDY OF EYE001 GIVEN BY INTRA-VITREOUS INJECTION TO BEAGLE DOGS.

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EYE001, an RNA oligonucleotide attached to a PEG group, inhibits angiogenesis in the retina of the eye by binding to vascular endothelial growth factor ($VEGF_{165}$). It has shown activity in Phase I clinical trails for age-related macular degeneration, a disease associated with excess choroidal neovascularization. EYE001 is unique, in that it is one of the first RNA-based products under clinical development to inhibit a mammalian growth factor. The objective of the study was to determine local tolerance and toxicity of EYE001 when administered once every 2 weeks to beagles *via* intra-vitreal injection for nine months. Animals received intra-vitreal injections of vehicle or EYE001 (0.1 ml volume at 3.0 mg/ml, 10.0 mg/ml or 30.0 mg/ml, into each eye). Dosage was calculated on the aptamer (RNA) component of the test article. While animals were under light anesthesia, an eyelid speculum was inserted to keep the eyelid open. The globe was rotated in an inferior fashion. The needle tip (27 gauge) of the syringe was placed perpendicular to the globe at a position 7 mm superior from the limbus on the scleral portion of the globe and pressure applied until the needle tip just penetrated the sclera. The angle to the orthoganol injection line was then increased (30-45°) by rotation downward to a point that facilitated administration of the material into the posterior portion of the eye. Possible toxicity was observed by eye exams using an indirect ophthalmoscope and slit lamp, fundus photography, electroretinographic and intraocular pressure measurements, daily macroscopic observation, clinical signs, body weight and food consumption determinations. Animals had blood and urine samples collected for hematology, coagulation and serum clinical chemistry profiles and semi-quantitative urinalyses prior to study initiation and at weeks 13 and 27. Through thirty weeks, the data indicate that EYE001 does not appear to cause local or systemic toxicity in the beagle dog.

688 PROTECTIVE ROLE OF THE HUMAN CORNEAL ALDH3A1 AGAINST UV-INDUCED TOXICITY.

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Aldehyde dehydrogenase 3A1 (ALDH3A1) is a significant constituent of mammalian corneal epithelium, representing 20-40% of total water soluble protein. Several hypotheses are proposed for the role of ALDH3A1 which include: (a) metabolism of cytotoxic aldehydes formed during UV-induced lipid peroxidation, such as 4-hydroxy-2-nonenal (4-HNE), (b) direct absorbance of UV-light, and (c) generation of NAD(P)H, which has recently been assigned a UVB absorbing species. The aim of this study was to develop human corneal epithelial cell lines expressing the human ALDH3A1 at levels similar to those observed *in vivo*, and to

study the role of this enzyme against UV-induced toxicity. We have established three types of transfected cell lines expressing ALDH3A1 at high, moderate and low levels. Our data indicate that ALDH3A1 protects against 4-HNE-induced cytotoxicity. Furthermore, Western blot analysis using antibodies against 4-HNE-adducted proteins revealed that ALDH3A1 also prevents adduction of the corneal epithelial proteins by 4-HNE. Enzyme kinetics with a human recombinant ALDH3A1 showed a Km of 32 mM for 4-HNE. Finally, ALDH3A1 expression protects corneal epithelial cells against UVB and/or UVC-induced cytotoxicity. In conclusion, the human ALDH3A1 appears to be a significant factor against UV-induced toxicity. [Supported by NEI 1 R29 EY11490].

689 INDUCIBLE EXPRESSION OF GLUTAMATE-CYSTEINE LIGASE AFFECTS CARBON TETRACHLORIDE-INDUCED LIVER INJURY IN TRANSGENIC MICE.

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Glutathione (GSH) is a major free radical scavenger and is important in detoxification of xenobiotics such as carbon tetrachloride (CCl4). The rate-limiting enzyme in GSH synthesis is glutamate-cysteine ligase (GCL) which is composed of catalytic (GCLc) and modifying (GCLm) subunits. Transgenic mice that conditionally overexpress GCLc and/or GLCm using a mifepristone (RU486) sensitive GeneSwitchTM system have been generated, and we have demonstrated RU486-inducible expression of the transgene(s) in the liver of these animals. We examined the influence of GCLc and GCLm overexpression on the extent of CCl4-induced liver toxicity. GCL transgenic mice were less susceptible to liver injury following RU486 administration and subsequent CCl4 treatment as revealed by serum ALT activity measurements and histopathology scores of the liver. GCL transgenic mice also showed lower levels of lipid peroxidation that is an early event in CCl4-induced liver injury. In contrast, administration of RU486 in nontransgenic mice synergized carbon tetrachloride-induced liver injury.

690 REGULATION OF THE MOUSE GLUTAMATE CYSTEINE LIGASE MODIFIER SUBUNIT GENE *VIA* THE ARE AND NRF/AP1 BINDING SITES.

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We previously reported that basal and BHQ-inducible expression of the mouse glutamate-cysteine ligase modifier subunit gene (Gclm) was dependent upon regulatory elements located within 787 bp upstream of its translation start codon. This region is highly homologous to the human GCLM promoter and contains a conserved antioxidant response element (ARE). Electromobility shift assays (EMSAs) reveal constitutive and BHQ-inducible binding of mouse Hepa-1 nuclear factors to this ARE, and mutagenesis of the ARE in a luciferase reporter construct containing 1.3 kb of Gclm promoter preferentially diminished inducible expression and affected basal expression as well. EMSAs reveal constitutive and BHQ-inducible binding of nuclear factors at a putative Nrf/AP1 site 90 bp upstream of the ARE. A single base-pair substitution at this site reduced basal expression of the reporter 100-fold while BHQ induction was conserved. The transcription factor Nrf1 mediates Gclm expression as evidenced by a 10-fold reduction in luciferase reporter activity in Nrf1-deficient cells which can be partially restored upon cotransfection with Nrf1 cDNA. Deletion constructs indicate that Nrf1-responsive elements lie within the region containing the ARE and upstream Nrf/AP1 site, and inclusion of a-Nrf1 or a-Nrf2 antibodies inhibits binding and induces a subshifted band respectively in binding reactions for both the ARE and Nrf/AP1 sites. Finally, mutations of the ARE in reporter constructs diminished basal and inducible expression in wild-type MEFs but had no effect in Nrf1-deficient cells. Taken together, these data indicate regulation of Gclm by the transcription factors Nrf1 and Nrf2 *via* the ARE and upstream Nrf/AP1 binding site.

691 GLUTAMATE CYSTEINE LIGASE LEVELS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTE SUBTYPES.

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Glutahione (GSH) plays a role in the immune response associated with inflammation. GSH disruption has been implicated in inflammatory lung diseases such as idiopathic pulmonary fibrosis, cystic fibrosis and chronic beryllium disease. Glutamate cysteine ligase (GCL) is the enzyme catalyzing the rate-limiting step in the formation of GSH. The aim of this investigation was to determine the levels of the GCL catalytic subunit (GCLC) in human peripheral blood lymphocyte (HPBL) subtypes according to cell cycle in normal healthy individuals. PHA stimulated HPBL GCLC levels were measured by staining with a rabbit polyclonal anti-GCLC peptide antibody and a goat anti-rabbit FITC labeled secondary antibody. HPBL subtypes and cell cycle phases were determined by staining for lymphocyte cell surface markers and DNA content. Antibody binding was then analyzed on a flow cytometer and a multiparameter analysis performed utilizing Multiplus software. PHA stimulated lymphocytes were found to have similar GCLC levels as non-stimulated lymphocytes. CD8 T-cells have the highest levels of GCLC. CD14 monocytes have the lowest GCLC levels which decrease further upon PHA stimulation. CD19 PHA stimulated B-cells have intermediate levels of GCLC which increase upon PHA stimulation. Cell cycle analyses suggest G1 phase GCLC levels are highest in CD4 and CD8 T-cells. Whereas, G1 and S/G2 phase GCLC levels are similar in CD14 monocytes. G1 phase GCLC levels in CD19 B-cells are higher in the PHA stimulated B-cells than in the non-stimulated B-cells. This information will contribute to the understanding of the role of GSH and GCL in the proliferation of lymphocyte subtypes and in defining potential immunotoxicity within sensitive populations. This study was supported by the Department of Energy *via* the Consortium for Risk Evaluation with Stakeholder Participation and NIH grants P30-ES04696, P50-ES07033

692 GENERATION OF A *Gclc(ii)* INDUCIBLE KNOCKOUT MOUSE LINE, USING THE *Cre/loxP* SYSTEM.

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Glutathione (GSH) is the most abundant nonprotein thiol in mammalian cells. Glutamate-cysteine ligase (GCL) is the rate-limiting enzyme in the glutathione (GSH) biosynthesis pathway. This holoenzyme is a heterodimer comprising a catalytic subunit (GCLC) and a modifer subunit (GCLM). Although *Gclc(-/-)* mice homozygous for the targeted disruption of the *Gclc* gene have provided insight into the developmental roles of GSH synthesis, the early embryonic lethality of these animals precludes studies of GSH during later development and organogenesis. To overcome this obstacle, we have generated an inducible knockout mouse line, whose *Gclc* gene was flanked by *loxP* sites in introns 3 and 6. Homozygous *Gclc(i/i)* mice containing the floxed *Gclc* gene have the same GSH level as *Gclc(+/+)* wild-type mice, suggesting that insertion of the *loxP* sites has no effect on *Gclc* expression. The ability of the *Cre* recombinase enzyme to cause deletion of the floxed allele was tested *in vivo* by crossing *Gclc(i/i)* mice to mice carrying a *Cre* transgene. Homozygous deletion of the floxed allele in the *Cre/loxP* progeny resulted in embryonic lethality that coincides with the phenotype observed in the conventional knockout mouse. This inducible knockout mouse line will serve as a valuable tool to investigate the role of GSH synthesis in a tissue- and temporal-specific manner.——*Supported, in part, by NIH grants P30 ES06096 and R01 AG09235.*

693 GLUTATHIONE S-TRANSFERASE EXPRESSION IN HUMAN LIVER MITOCHONDRIA.

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The mitochondrial environment is rich in reactive oxygen species (ROS) that may ultimately peroxidize membrane proteins and generate highly reactive unsaturated aldehydes such as 4-hydroxy-2-nonenal (4HNE). Accordingly, multiple protective pathways have arisen to ensure mitochondrial function during oxidative stress. In the present study, affinity chromatography was used to isolate glutathione S-transferases (GSTs) from human hepatic mitochondria. HPLC-subunit analysis of affinity-purified liver mitochondria suggested the presence of at least two or more mitochondrial glutathione S-transferases (GSTs). Amino acid sequencing and immunologic analyses of affinity-purified GST indicated the presence of two alpha class GSTs related to hGSTA4-4 and hGSTA1/2, isozymes that protect against oxidative injury. Matrix-assisted laser desorption/ionization mass spectroscopy was used to further characterize the mitochondrial GST isozymes. Results from this ongoing investigation suggest that GST may represent an important pathway to protect the mitochondrion from the deleterious effects of ROS. Supported by N.I.H. ES-09427 and U.S.E.P.A. R-827441.

694 OVEREXPRESSION OF A MITOCHONDRIAL GLUTATHIONE TRANSPORTER IN NRK-52E CELLS.

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The dicarboxylate carrier (DCC) of the mitochondrial inner membrane was cloned from total rat kidney RNA, expressed in *E. Coli* as a His_6-tagged protein, and shown to transport both malonate and glutathione (GSH) with properties that

were similar to what we described previously. The cloned, expressed, purified and reconstituted DCC protein exhibited saturable uptake of both malonate and GSH. Uptake of GSH was inhibited by several competitive substrates (butylmalonate, L-γ-glutamyl-L-glutamate, ophthalmic acid, malonate) and by pyridoxal 5'-phosphate and mercurials. Malonate uptake was inhibited by butylmalonate, pyridoxal 5'-phosphate, mercurials and, most significantly, by GSH. Like many immortalized cell lines, NRK-52E cells (derived from normal rat kidney cells) exhibited low, endogenous levels of many mitochondrial processes, including respiration, activities of dehydrogenases, and substrate transport. Fluorescent imaging with MitoTracker® Orange suggested, however, that these cells do contain a high density of mitochondria; we conclude that many mitochondrial processes are, therefore, down-regulated. NRK-52E cells were transiently transfected with the cDNA for rat DCC to overexpress mitochondrial GSH transport activity. Expression of the rat DCC-His_6 fusion protein was confirmed by Western blot analysis. Mitochondria from transfectants exhibited 2.1 to 6.8-fold higher activity for uptake of 5 mM GSH and 2.0 to 10.7-fold higher activity for uptake of 1 mM malonate as compared to mitochondria from normal cells. We are currently developing stable transfectants that overexpress the DCC protein and investigating the effect of higher mitochondrial GSH transport activity in the transfected cells on susceptibility to chemically induced apoptosis. Preliminary results suggest that transfectants are indeed less sensitive than normal cells to chemically induced injury. (Supported by NIDDK Grant DK40725.)

695 ALKYLATION AND INACTIVATION OF HUMAN GLUTATHIONE TRANSFERASE ZETA (HGSTZ1-1) BY MALEYLACETONE AND FUMARYLACETONE.

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Glutathione transferase zeta (GSTZ1-1) catalyzes the cis-trans isomerization of maleylacetoacetate (MAA) or maleylacetone (MA) to fumarylacetoacetate or fumarylacetone (FA), respectively. GSTZ1 also catalyzes the glutathione (GSH)-dependent biotransformation of a variety of α-haloacids, including dichloroacetic acid (DCA). This study was designed to investigate the mechanism of inactivation of hGSTZ1-1 by MA and FA and to determine the covalent modification of hGSTZ1-1 by MA and FA in the presence and absence of GSH. MA and FA (0.01-1 mM) inactivated hGSTZ1-1 in a concentration- and time-dependent manner and these effects were blocked by GSH. MA or FA did not, however, inactivate the C16A mutant of hGSTZ1c-1c. ESI-MS/MS and SALSA (Scoring Algorithm for Spectral Analysis) analyses of tryptic digests of hGSTZ1 variants revealed that the active site (SSC*SWR) and C-terminal (LLVLEAFQVSHPC*R) cysteine residues of hGSTZ1-1 were modified by Michael addition reactions. MA and FA ($HOOCCH{=}CHCOCH_2COCH_3$) adduction caused diagnostic 156 Da shifts in the masses of the modified peptide ions and in their MS-MS fragment ions. Alkylation of the active site cysteine residues, but not of the C-terminal cysteine, was relatively less intense when hGSTZ1-1 variants were incubated with MA or FA in the presence of S-methyl glutathione. These data indicate that MA and FA are substrate and product inactivators of GSTZ1-1 and covalently modify GSTZ1-1 at the active site cysteine residue in the absence of GSH. That these effects are blocked by GSH indicates that the mechanism of catalysis of GSTZ1-1 is by an obligate ordered reaction with GSH binding before MAA or MA. These data also indicate that MA and FA may covalently modify and inactivate other proteins that have accessible cysteine residues and thereby in part contribute to DCA- or hypertyrosinemia type I-associated toxicities. (Supported by NIEHS grant ES03127, ES10056, and ES06694).

696 DEPENDENCE OF CYTOLOGICAL GRADINGS OF EXFOLIATED UROTHELIAL CELLS UPON GSTM1 AND GSTT1 POLYMORPHISM IN FORMERLY BENZIDINE-EXPOSED WORKERS.

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The distribution of the polymorphic alleles of the genes coding for glutathione S-transferases (GSTs) M1 and T1 was compared to results of a cytological grading according to Papanicolaou of exfoliated urothelial cells in a non-diseased high-risk group formerly exposed to benzidine in the Shanghai dyestuff industry (n=317). All subjects were genotyped for GSTT1 and M1 gene polymorphism by allele-specific PCR. Individuals were stratified according to the job and duration of exposure. A subgroup of 78 cases with cytological gradings grade III or higher (according to Papanicolaou) showed a significant under-representation of the combination of GSTT1 0/0 and M1 0/0 genotypes compared to 238 subjects with a cytological classification lower than grade III (OR=0.55, 95% CI=0.31-0.98, P=0.04). These results suggest that neither the GSTM1 0/0 or GSTT1 0/0 genotypes alone nor their combination do have a clear association with cytopathological changes in exfoliated urothelial cells from individuals previously exposed to benzidine in Shanghai. This is contradictory to studies reporting that the GSTM1 0/0 genotype is associated with an increased risk for bladder cancer in the general population, mostly outside China.

697 CELLULAR GLUTATHIONE DISTRIBUTION DURING OXIDATIVE STRESS.

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Oxidative stress causes molecular changes within cells that can lead to cytotoxic outcomes. Glutathione, a ubiquitous thiol-reactive tripeptide, plays a major role in regulating responses to oxidants. Glutathione in its reduced form (GSH) also is suggested to assist in intracellular protein translation and trafficking. With the use of a monoclonal antibody (mouse IgG1; 8.1GSH; StressGen, Victoria) against the glutathione adduct with N-ethylmaleimide, we have determined the cellular distribution of GSH in three human leukocyte cell lines (Jurkat, T cells; Daudi, B cells, and THP-1, monocyte-like) by an immunoelectron microscopy method (gold conjugated goat anti-mouse IgG). In general, the distribution pattern was quite similar with GSH distributed in most cellular compartments, including the nucleus. The lumen of rough endoplastic reticulum was the one area with less GSH. After stress with diamide (0.1 mM), ethycrinic acid (0.1 mM), or L-buthionine-(S, R)-sulfoximine (BSO; 1 mM), the distribution patterns among the cell types demonstrated some differences. On the whole, the mitochondria maintained residual reservoirs of GSH. The THP-1 line showed a major increase in vacuoles and membrane blebbing; however, results were not consistent even within this homogenous cell line, suggesting possible cell cycle influences. In general, the immunoelectron microscopy results were similar to results by flow cytometry with regard to the amount of 8.1GSH-reactive GSH in the various cell lines after the oxidative stresses. Work support, in part, by NIEHS grant ES03778.

698 NUCLEAR GLUTATHIONE REDUCTASE ACTIVITIES ARE INCREASED DURING S PHASE OF CELL CYCLE IN CHO CELLS.

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Glutathione reductase (GR) is a key enzyme in the glutathione antioxidant pathway, reducing glutathione disulfide (GSSG) to the thiol form (GSH). We have previously demonstrated GR localization in the mitochondria, the nucleus, and the nucleoli of rat liver tissue. The goal of the present study was to test the hypothesis that nuclear GR activities changed in a cell cycle-dependent manner, which would suggest a role for GR in regulation of cell cycle progression and related cell responses. CHO (Chinese Hamster Ovary) cells were plated at 3×10^6 cells/plate in McCoy's media supplemented with 10% FCS and allowed to adhere for 24 h. The media was then replaced with McCoy's media supplemented with 0.1% FCS for 72 h prior to experiments allowing the cells to arrest in G0. At 0 h, the CHO cells were re-fed McCoy's media with 10% FCS and were harvested at 0, 4, 16, and 20 h. Cells from each time point were harvested for subcellular fractionation using the digitonin solubilization method of Bronfman [Anal Biochem 255:252], and nuclei and mitochondria were separated by centrifugation. Additional cells were fixed with ethanol and stained subsequently with propidium iodide for flow cytometric analysis, to determine the degree of synchrony and the stage of cell cycle. Flow cytometry indicated that 65-75% of the cells had arrested at G0 at the 0 h time point, by 16 h had moved into S phase, and by 20 h were in G2/M phase. GR activities in the whole cell homogenates, cytoplasm, and the mitochondria were not different during cell cycle progression. Nuclear GR activities, expressed as mU/mg protein, were 1.4±0.32 in G0, 3.08±1.35 in G1, 9.70±0.80 in S, and 3.92±2.17 in G2/M, indicating a marked increase in GR activity at S phase, $p<0.05$. The data indicate that nuclear GR activities change as cells progress through cell cycle and implicate a role for redox regulation of cell cycle progression. Supported by GM44263 from the NIH.

699 EFFECTS OF FASTING ON TISSUE CONTENTS OF COENZYME A AND RELATED INTERMEDIATES.

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Exposure of rats and mice to hyperoxia decreases lung coenzyme A (CoASH) contents, with a decrease of 50 % observed in adult male Fischer-344 rats exposed to >95 % O2 for 48 h. The decreases in lung CoASH levels are not accompanied by

increases in contents of the mixed disulfide with GSH (CoASSG), as might be expected of a primary oxidative stress on CoASH status. Animals exposed to hyperoxia exhibit decreased food intake, and the present studies were to test the hypothesis that fasting would decrease lung CoASH contents, thereby suggesting a mechanism for the effects of hyperoxia. Adult male Fischer-344 rats were examined after 0, 24 or 48 h of fasting (n=5, 6, 6, respectively). Fasting for 24 or 48 h did not affect (1-way ANOVA, Student-Newman-Keuls) lung CoASH levels or lung weights, despite 6 and 12 % losses in body weights. Lung GSH concentrations (nmol/g tissue) and contents (nmol/whole organ) and GSSG contents were lower in rats fasted for 48 h than in fed rats. Liver weights and GSH and GSSG contents and concentrations were lower in rats fasted for 24 or 48 h than in fed rats. Hepatic CoASH concentrations increased during fasting, but hepatic contents of CoASH remained remarkably constant. Liver protein contents (mg/whole organ) decreased after 24 and 48 h, but protein concentrations (mg/g tissue) were higher in rats fasted 48 h than in fed rats. Overall, GSH, GSSG, and protein contents in liver and skeletal muscle, decreased with fasting, but significant changes in CoASH levels were not observed. Diminished food intake in animals exposed to hyperoxia may contribute to effects on GSH and GSSG contents, but does not explain the effects of hyperoxia on lung CoASH contents. CoASH and derived thioesters participate in many cellular functions, and if CoASH depletion proves to be relevant to lung injury caused by hyperoxia, dietary supplementation or support of mechanisms needed to sustain CoA levels could be helpful in prematurely born infants as well as in adults.

700 THE NATURE OF PROTEIN DISULFIDE ISOMERASE ALKYLATION BY THE EPISULFONIUM ION DERIVED FROM S-(2-CHLOROETHYL)GLUTATHIONE.

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Glutathione (GSH) protects cells although GSH conjugation can cause cytotoxicity by enhancing the reactivity of some xenobiotic chemicals, i.e., halogenated alkenes, quinines, and isothiocyanates. GSH conjugation to form S-(2-chloroethyl)glutathione (CEG) as a consequence of 1, 2-dichloroethane (DCE) exposure is another example of a toxication reaction. CEG can form an electrophilic episulfonium ion that can react with specific sites in DNA and proteins. Evidence indicates that mutagenicity of DCE is due to the GSH conjugate. Our experiments are based on the reactivity of this episulfonium ion with proteins of the thioredoxin family (i.e., thioredoxin (TRX) and protein disulfide isomerase(PDI)). Mammalian PDI has two thioredoxin fold active sites each containing two cysteines. When reduced, these four cysteines are expected to be the major targets of alkylation by the episulfonium ion of CEG, preferring the first cysteine in each active site sequence if PDI alkylation follows the same pattern observed with TRX. Reduced recombinant rat PDI was incubated for 90 minutes with a range of CEG concentrations from equimolar to 50-fold excess CEG. Based on ESI-MS and MALDI-MS of the whole protein before and after alkylation, 50% of the protein is either monoalkylated (38%) or bisalkylated (12%). Further increasing CEG concentrations shows a rapid decrease in native PDI to non-detectable amounts in 50 fold CEG excess. Alkylation of PDI does not correlate with an inhibition of reductase or isomerase activity as well as the alkylation of TRX. Iodoacetamide carboxy methylation of PDI inhibits activity suggesting that the CEG derived episulfonium ion may not be as accessible to the active sites of PDI as is observed with TRX. Peptide mapping of alkylated PDI and further sequencing of modified peptides by ESI-MS/MS is underway. (This work was supported by grants from the NIEHS (ES00040 and ES00210).

701 POSTEXPOSURE TREATMENT WITH 2-MERCAPTOETHANE SULFONIC ACID (MESNA) INCREASES THE SURVIVAL OF MICE FOLLOWING EXPOSURE TO PHOSGENE.

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Phosgene (CG) is a highly reactive oxidizing/acylating gas used industrially as a chemical intermediate for the production of pharmaceutical drugs, pesticides, and polyfoam rubber products. CG can cause life-threatening pulmonary edema by reacting with peripheral lung conpartment tissue components. The insidiousness of CG inhalation is that there is a clinical latent phase where edema is not usually evident until 6-36 hr after exposure. Treatment modalities have primarily relied on pretreatments. This study was designed to investigate the effect of a clinically available sulfhydryl donor, MESNA. On postexposure survival rates and pulmonary edema formation (PEF) in mice. Seventy-two male mice were exposed to 32 mg/m3 (8ppm) CG for 20 min followed by room air washout for 5 min. Four groups of 18 mice each were injected i.p. at 20 min and 3 hr after exposure with 0 (saline), 5, 25, or 50 mg/kg MESNA. Within 12 hr of exposure gravimetric analysis was performed on lung tissue dry weights (DW), wet weight (WW), and wet/dry weight ratio (W/D). Survival rates (SR) were also determined at both 12 and 24 hr postexposure. Both 5 and 25 mg/kg MESNA had no positive effect on any parameter measured. However, at 12 hr the 50 mg/kg dose showed a marginal decrease in lung tissue WW compared with 0 mg/kg, 118+/-10 mg vs 135+/-9 mg ($P<0.07$), respectively. MESNA, 50 mg/kg, also significantly decreased the W/D ratio, 7.2+/-0.5, vs 0 mg/kg, 9+/- ($P<0.038$) also at 12 hr. SR for the 50 mg/kg mice was 72% at 12 hr, $P<0.07$ (chi square) compared with 44% for 0 mg/kg. The 24 hr SRs were 50% and 33%, respectively. Treatment with 50 mg/kg MESNA also significantly enhanced lung tissue protective nonprotein sulfhyudryl levels, 21+/-2 uM/kg vs 0 mg/kg MESNA, 2.9+/-0.1 ($P<0.0001$) and reduced lung tissue protein oxidation, 5.2+/-1 vs 14.8+/-1 uM($P<0.0001$), at 12 hr. We conclude that the sulfydryl donor, MESNA, may be useful as a therapeutic agent against phosgene-induced acute lung injury by ameliorating PEF and reducing tissue oxidation.

702 ACROLEIN DEPLETES GLUTATHIONE AND THIOREDOXIN IN HUMAN BRONCHIOLAR CANCER CELLS.

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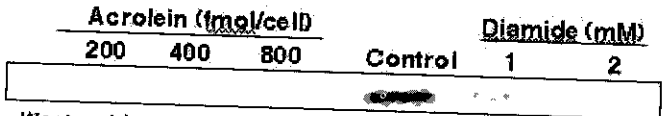
Glutathione (GSH) and thioredoxin (Trx) are important components of the cellular thiol redox system. Both play vital roles in regulating the activity of various transcription factors such as NF-κB and AP-1 that are involved in cell growth and cell death. Acrolein is a highly reactive α, β-unsaturated aldehyde commonly found in the environment, particularly as a component of smoke. Acrolein's toxicity is related to its electrophilic nature, and appears to correlate with GSH depletion. However, the effects of acrolein on other cellular thiols is less well-known. The current data demonstrate with biotin maleimide detection of free sulfhydryls on PVDF membranes that, beginning 1 h after treatment of H727 human bronchiolar cancer cells with 100fmol/cell acrolein or more, there is an overall decrease in protein thiols. In addition, treating H727 cells for 0.5 h with the same doses of acrolein depletes GSH levels in a dose-dependent manner and causes a loss in Trx protein levels. The thiol oxidant diamide also caused a loss in Trx protein. These acrolein-mediated changes in GSH and Trx are likely to disrupt the activity of various transcription factors and, in conjunction with an inhibition of caspase activities, may be important in the necrotic (not apoptotic) cell death caused by this aldehyde. (Supported by RO1 ES09791 and Center Grant ES07784.)



Western blot of thioredoxin using 50 µg of a H727 cell lysate. Treatments were for 0.5 h.

703 EFFECT OF 2, 4-HEXADIENAL ON TISSUE GLUTATHIONE AND MALONDIALDEHYDE LEVELS IN FISCHER 344 RATS.

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2, 4-Hexadienal (2, 4-HX) is an α, β-unsaturated aldehyde formed by peroxidation of unsaturated lipids that causes neoplasms in the forestomach of rats. The objective of this study was to investigate the mechanism of action of this agent. 2, 4-HX was administered orally in corn oil to male and female Fischer 344 rats for 10 days. Plasma, liver, and forestomach samples were collected and analyzed for malondialdehyde (MDA) and glutathione (GSH and GSSG) by HPLC with fluorescence detection. No mortality, clinical signs or body weight changes were noted in rats administered either 90 or 120 mg/kg/day 2, 4-HX. Marked changes in the forestomach were observed microscopically including chronic inflammation, acanthosis, hyperkeratosis, ulceration and squamous epithelial cell papilloma; the severity was dose dependent. Significant differences in MDA content of plasma, liver or forestomach were not observed in the treatment groups compared to the vehicle or untreated groups, except for plasma MDA, which was slighrly decreased in rats treated with 120 mg/kg/day. There was no apparent effect of either dose of 2, 4-HX on plasma levels of GSH. In the liver, the total amount of GSH was decreased with increasing dose of 2, 4-HX at 1 and 4 hr post-dose, but by 24 hr post-dose the total GSH levels returned to those observed in the vehicle controls. In contrast, 2, 4-HX administration increased the total amount of GSH in the forestomach, and at 1 hr and 4 hr post-dose this was largely due to increased GSSG; by 24 hr forestomach GSH levels had also become elevated. The GSH redox status in forestomach samples was decreased by administration of 2, 4-HX. In summary, 2, 4-HX treatment

for 10 days alters the tissue glutathione levels in both a target tissue, the forestomach, causing a compensatory increase in total glutathione and a decrease in the redox status, and in a non-target, the liver, in which total glutathione was significantly reduced. (This work was supported by NIEHS contract N01-ES-95437.)

704 ACUTE EFFECTS OF DIQUAT ON REDOX STATUS OF HEPATIC COENZYME A AND GLUTATHIONE IN FISCHER-344 RATS.

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Exposures to oxidant stresses *in vivo* frequently are characterized by measurements of glutathione disulfide (GSSG), but initiation of irreversible injury has correlated poorly with tissue concentrations of GSSG. Because 70% or more of hepatic coenzyme A is intramitochondrial and CoASH and CoASSG undergo thiol-disulfide exchange reactions with GSH and GSSG, we measured tissue concentrations of CoASH and CoASSG, to assess possible compartmental effects in the oxidant stress responses initiated by administration of diquat. Adult male Fischer-344 rats were given 0, 0.05, or 0.1 mmol/kg of diquat, i.p. After 2, 4, 6, or 24 h, the animals were anesthetized with pentobarbital, blood drawn, and livers freeze-clamped. Plasma ALT activities were measured as assessments of liver damage, concentrations of CoASH and CoASSG were measured by HPLC, and hepatic GSH and GSSG concentrations were measured by enzyme recycling methods, as we have described previously. Diquat administration produced dose-dependent increases in ALT activities, but did not decrease hepatic concentrations of CoASH (123 nmol/g of liver in controls), in contrast with the effects of hepatotoxic doses of acetaminophen [Chem Res Toxicol 13:873, 2000]. Diquat caused dose-dependent increases in hepatic CoASSG levels that showed significant correlations with plasma ALT activities (P=0.0016), also in contrast with the effects observed with acetaminophen-initiated hepatic injury; however, in no animal did the hepatic CoASSG concentrations exceed 10 % of the CoASH content. Hepatic GSSG concentrations showed dose-dependent increases and correlated with elevations in plasma ALT activities, although the mean levels in diquat-treated rats were little more than twice the levels observed in saline-treated controls. The present data document effects of diquat on hepatic CoASH/CoASSG redox status, but do not suggest marked mitochondrial compartmentalization of diquat-induced oxidant stress responses *in vivo*. Supported by GM44263 from the National Institutes of Health.

705 IMMUNOTOXICITY OF 2-BROMOPROPANE AND 1, 2-DIBROMOPROPANE IN MICE AND RATS.

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2-Bromopropane (2BP) is a major component of the mixture of SPG-6AR and Solvent 5200 which is a substitute of chlorofluorocarbon. In 1995, an occupational exposure to 2-BP has been a social issue in Korea. Many female workers in an electronic company were found to have amenorrhea and male workers were diagnosed with oligospermia. In the present studies, immunotoxic effects of 2-BP were investigated in male Sprague-Dawley rats and female BALB/c mice. The rats were treated po with 2-BP at 100, 330 and 1000 mg/kg for 28 days. Four days before necropsy, the rats were immunized iv with sheep red blood cells (SRBCs). The body and thymus weights were reduced by 2-BP at 1000 mg/kg. In addition, the numbers of splenic and thymic cells were decreased by 2-BP. In hematology, the number of WBCs, RBCs and platelets was reduced. Among the serum clinical parameters, chloride ion was increased by 2-BP. The antibody response to SRBCs was suppressed at 1000 mg/kg. With immunized animals, immunophenotyping of splenic and thymic cells was performed to investigate the changes of the number of macrophages, B- and T-cells in spleen and the number of $CD4^+$ and $CD8^+$ cells in thymus. The numbers of most cell types were decreased in the spleen when animals were treated with 2-BP at 1000 mg/kg. Likewise, all cell types of thymus were decreased by 2-BP. In a subsequent study, effects of 2-BP and 1, 2-dibromopropane (1, 2-DBP) on the antibody response were compared in mice. When mice were treated po with either one of these chemicals for 7 days, the antibody response was suppressed only by 1, 2-DBP. The present results suggest that 2-BP may have an immunotoxic potential in rats, and that 1, 2-DBP contained in the Solvent 5200 may contribute to the immunotoxicity, although 2-BP is a major component. (Supported by grant No. 2000-2-21800-001-3 from KOSEF).

706 ETHANOL ALTERS POLY I:C INDUCED NK CELL TRAFFICKING AND ACTIVATION.

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Natural killer (NK) cells are critical in resistance to lung tumor establishment in a B16F10 tumor model using female B6C3F1 mice. Polyinosinic:polycytidylic acid (poly I:C, 0.1 mg, i.p.) activation of NK cells increases elimination of tumor nodules. This effect is reduced following oral gavage of ethanol (EtOH, 6 g/kg). This study determined if decreased resistance is due to altered trafficking (flow cytometry) and/or NK cell activation (chromium release assay) in the spleen and lungs 4 and 12 h after EtOH and poly I:C administration. Initial studies using anti-NK1.1- (0.5 mg, i.p.) mediated NK cell depletion *in vivo* 48 h before and 0, 6, 12, and 24 h after B16F10 tumor injection ($4x10^5$, i.v.) demonstrated that the majority of tumor cells are cleared by NK cells in 12 h ($p<0.001$). Flow cytometry revealed that the percentage of NK cells in the lungs at 4 h (6±1%, mean±SEM) was equivalent after vehicle, poly I:C and EtOH/poly I:C treatment. At 12 h, however, an increase induced by poly I:C treatment (from 4±.1 to 6±1%, $p<0.001$) was abrogated by EtOH (4±1%, $p<0.01$). Corresponding normalized (poly I:C treatment as 100%) NK cell lytic function in the lungs was increased from 26±6 to 100±15% ($p<0.001$) and 11±1 to 100±7% ($p<0.05$) following poly I:C treatment at 4 and 12 h, respectively. EtOH reduced this function to 77±21% (ns) at 4 h and 60±8% ($p<0.001$) at 12 h. Similar analysis of splenocytes revealed no differences in percentage of NK cells at 4 (5±1) or 12 h (5±1). Lytic function, however, increased from 17±2 to 100±17% ($p<0.01$) at 4 h and 15±2 to 100±7% ($p<0.001$) at 12 h. EtOH/poly I:C treatment resulted in decreases to 57±16% ($p<0.05$) at 4 and 55±7% ($p<0.001$) at 12 h. These data indicate that EtOH decreases trafficking of NK cells into the lungs 12 h after poly I:C administration, accompanied by reduction in lytic function. Although EtOH did not alter numbers of NK cells in the spleen, it decreased lytic function by half at both 4 and 12 h. This suggests that while EtOH can alter trafficking, its affects NK cells primarily by limiting their activation.

707 IMMUNE STIMULATION FOLLOWING TREATMENT WITH THE HERBAL MEDICINE ECHINACEA PURPUREA IN FEMALE B6C3F1 MICE.

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Echinacea Purpurea (EP) is one of the few herbal medicinal plants indigenous to North America. For hundreds of years it has been used by American Indians to treat various aliments. As part of a comprehensive program of the National Toxicology Program, we have been evaluating the water/alcohol extract of EP for its ability to modulate the immune system. Female B6C3F1 mice were administered EP daily by oral gavage in a 0.5% methylcellulose vehicle at doses of 30, 100, 300, 600, or 1000 mg/kg for 28 days in range-finding studies and at 300, 600, or 1000 mg/kg in more comprehensive protocol studies. Overall, there were no remarkable effects on the toxicological parameters including, body weight, selected organ weights, or hematological parameters. Similarly, minimal immunological effects were observed. Parameters not affected included, splenocyte differential, mixed leukocyte response (MLR), and natural killer (NK) cell activity. Increases were observed in the spleen IgM antibody-forming cell (AFC) response to sheep erythrocytes at all dose levels; however, the response was not dose dependent and not always statistically significant. Enhanced T cell proliferation was observed following stimulation with anti-CD3 antibody in EP-treated animals. Furthermore, enhanced cytotoxic T lymphocyte (CTL) activity was observed at all effector-to-target ratios in the animals administered 300 mg/kg of EP, while the responses of animals treated with the higher doses of EP were not statistically different than the vehicle controls. Ongoing host resistance studies should help establish if the increases in the various components of the immune system stimulated by EP are sufficient to provide protection from various pathogens. (Supported by NIEHS Contract ES 55387).

708 COMPARISONS OF JP-8-INDUCED IMMUNE ALTERATIONS FOLLOWING TWO DIFFERENT ROUTES OF EXPOSURE IN FEMALE B6C3F1 MICE.

D. E. Keil, K. Scharstein, J. EuDaly and M. M. Peden-Adams. *Medical University of SC, Charleston, SC.*

JP-8 jet fuel is used widely in the military and commercial airline environments. Recent reports have implicated jet fuel as an acute immune and respiratory toxicant. Studies *via* inhalation and dermal exposures have reported similar results; however, oral exposure to jet fuel results in slightly different findings. Previously, no studies have assessed parallel exposures of JP-8 *via* different routes of administration. In this study female, B6C3F1 mice were exposed to JP-8 jet fuel either orally or dermally for 7 days. The oral dose used was 2000 mg/kg and was administered in a vehicle of olive oil. The dermal dose regime consisted of the application of 50 ul of neat JP-8, an exposure similar to previously published dermal studies. Two control groups were included for the dermal exposure; a group that was administered 50 ul of acetone and another with 50 ul of olive oil. Endpoints assessed included splenic and thymic weight and cellularity, liver weight, uterine wet weight, lymphocyte proliferation, natural killer cell activity, T-cell subpopulations, and the antibody plaque forming cell response. Decreases in thymus weight and cellularity were observed following oral exposure but not following dermal exposure. Liver weights

were increased following oral exposure but not following dermal exposure. Uterine wet weight was decreased by both exposure routes but this was not significantly different from the oil control. No effects were observed in NK-cell activity or mitogen-induced lymphocyte proliferation following the exposures. Splenic T-cell subpopulations were altered following dermal exposure. Suppression of the PFC response was observed in the oral JP-8 treatment as compared to its respective olive oil control. Additionally, the PFC cell response was suppressed following dermal exposure as compared to the oil control but not compared to the acetone control. Therefore, the varied findings regarding JP-8 immunotoxicity may be explained in part by the route of administration, as well as the types of controls used in reported studies.

709 SULFLURAMID-INDUCED ALTERATIONS IN IMMUNOTOXICOLOGICAL PARAMETERS.

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Fluorinated organic compounds have been manufactured for over 50 years and are currently used throughout industry in semiconductors for computers and other electronic equipment, as stain and water repellents, in floor waxes, popcorn bags, firefighting foam, denture cleaners, carpet spot cleaners, pharmaceuticals, and as pesticides. Manufacture and use of these compounds has steadily increased since the 1970s. Studies show that this class of compounds cause peroxisomal proliferation, hepatomegaly, altered steroidogenesis, and body weight decreases that are associated with a wasting syndrome; however, effects on immune function have not been assessed. This study utilized sulfluramid, a perfluorinated pesticide, as a representative compound from this class of chemicals. Adult female B6C3F1 mice were exposed *via* gavage to either the oil control or sulfluramid (1, 3, 10, or 30mg/kg) for 14 days. Significant decreases in body weight at the high dose (30 mg/kg/day) and increased liver/body weight ratios at 10 and 30 mg/kg/day were noted. Additionally, spleen and thymus weights and cellularity were greatly reduced. Although no differences were observed in natural killer cell activity or mitogen-induced lymphocyte proliferation, suppression was noted in the plaque forming cell (PFC) response and decreases were observed in thymic T-cell subpopulations. These data indicate that sulfluramid may be a potent immunotoxicant. Further studies are needed to fully characterize the extent of immune parameters effected and the role of the parent compound versues the known metabolite PFOS.

710 DDT INHIBITS FUNCTIONAL ACTIVATION OF MURINE MACROPHAGES AND DECREASES RESISTANCE TO INFECTION BY MYCOBACTERIUM MICROTI.

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DDT is still widely used in several parts of the world to control malaria, typhoid and dengue vectors. DDT has been shown to have immunotoxic effects in mice and to increase susceptibility to intracellular pathogens such as Mycobacterium leprae. However, little is known about the mechanisms underlying this effect. Activated macrophages play an important defensive role against intracellular pathogens, therefore our objective was to evaluate the effect of *in vitro* exposure to technical grade DDT, p, p'-DDT, p, p'-DDE and p, p'-DDD on the functional activation of J774A.1 macrophages and their capability to limit growth of intracellular pathogens, using Mycobacterium microti as a model. We evaluated cytotoxicity, cell proliferation, functional macrophage activity ($NO^{\bullet}$ and O_2^- production, and mRNA expression of TNF-*, IL-1* and iNO Synthase), and the ability of treated cells to limit infection by M. microti in IFN* activated macrophages exposed to 2.5, 5.0 and 10 µg/ml of DDT compounds. Doses of 5 and 10 µg/ml induced direct cytotoxic effects precluding meaningful analysis of the above parameters, whereas 2.5 µg/ml of all DDT compounds inhibited macrophage activity and reduced their ability to limit the intracellular growth of M.microti without inducing cytotoxicity. Technical grade DDT and p, p'-DDE were the more potent compounds. Therefore, exposure to DDT compounds could represent an important risk for infection development by those intracellular pathogens against which $NO^{\bullet}$ and/or O_2^- production represent the main immune protective mechanism.

711 ESTROGEN INDUCES THYMIC ATROPHY THROUGH INHIBITION OF CELL CYCLE.

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Estrogen is a modulator of the immune response. One of the main targets for estrogen in immune system is the thymus. Although recent research suggests that estrogen may inhibit thymocytes at different development steps, the mechanism(s) by which estrogen induces thymic atrophy is still unclear. In the present study, we have determined that inhibition of cell proliferation is one such mechanism. Mice were injected at various times with estradiol (E2) and then BrdU was injected 2 hours before mice were sacrificed. Thymocytes were isolated and stained with fluorescence conjugated antibodies for cell surface markers and BrdU. Data was acquired using a FACSCalibur flow cytometer with two lasers for four-color analysis. Our results showed that E2 induced thymic atrophy and reduced the numbers of thymocytes in all CD4+, CD8+, CD4+CD8+ (DP) and CD4-CD8- (DN) subsets. In analysis for cell proliferation, we found that E2 could inhibit DNA synthesis with BrdU incorporation in thymocytes and this effect was mainly in CD3-CD4+CD8+ DP and CD3-CD4-CD8- triple negative (TN) thymocytes. Furthermore, within the CD3-CD4-CD8- TN subpopulation, E2 mainly inhibited cell proliferation at CD44-CD25+ and CD44-CD25- stages. A time course analysis showed that inhibition induced by E2 appeared as early as 1 day after exposure, whereas the number of total thymocytes was not affected at this time point. The proliferation activity in DP cells returned to normal level, but was still inhibited in TN cells at day 10 after exposure. We also determined the effects of E2 in SCID mice, whose thymuses only contain immature CD4-CD8- DN cells, allowing us to focus our study in the earliest development stages of thymocytes. Our results confirmed that inhibition of cell proliferation was in CD44-CD25+ stage in SCID mice. By using a DNA PI staining for cell cycle and flow cytometry analysis, our results indicated that E2 could inhibit cell cycle from G0/1 phase entry into S/G2M phase. Taken together, our results clearly suggest that estrogen induced thymic atrophy is mainly through inhibition of cell proliferation.

712 DIFFERENTIAL INDUCTION OF APOPTOSIS IN MURINE LYMPHOID SUBPOPULATIONS *IN VIVO* FOLLOWING CO-EXPOSURE TO LIPOPOLYSACCHARIDE AND VOMITOXIN.

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Lipopolysaccharide (LPS) and vomitoxin (VT, deoxynivalenol) synergistically induce cell death at 12 hr in lymphoid tissue of the mouse, whereas the effects of these toxins alone were negligible. The purpose of this study was to assess the effects of LPS and VT co-exposure on apoptosis within specific T and B lymphocytes subsets in thymus, Peyer's patches, and bone marrow. Flow cytometry revealed that a single dose of LPS (0.1 mg/kg body, weight, po) together with VT (12.5 mg/kg body weight, po) resulted in apoptotic death of immature thymocytes ($CD4^-CD8^-$, $CD4^+CD8^+$) and mature thymocytes ($CD8^+$) in thymus with a concurrent loss of cell numbers. RU 486, a glucocorticoid receptor antagonist, significantly abrogated apoptosis in $CD4^-CD8^-$, $CD4^+CD8^+$ and $CD8^+$ subsets and also prevented loss in cell numbers. In Peyer's patches and bone marrow, $B220^+IgM^-IgD^+$ pro-B cells underwent apoptosis at 12 hr after toxin co-treatment. RU 486 blocked apoptosis in $B220^+IgM^-IgD^+$ subset, however, reduction of cell numbers was still observed at this time point. Taken together, these data suggest that LPS can interact with VT to induce the glucocorticoid-dependent apoptotic loss of immature thymocytes, cytotoxic T lymphocytes and pro-B cells in mouse lymphoid organs.

713 IMMUNOMODULATORY EFFECTS ASSOCIATED WITH ANTI TUMOR NECROSIS FACTOR (TNF) ANTIBODY TREATMENT IN THE FEMALE B6C3F1 MOUSE.

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Tumor Necrosis Factor Alpha (TNF-α) is responsible for stimulating numerous biologic functions including the inflammatory response, as well as enhancing the production of other cytokines involved in both immune and non-immune processes. Inhibition of TNF-α has been shown to have therapeutic benefits for the treatment of rheumatoid arthritis by reducing the associated inflammation and tissue degradation. Recently clinical data from inhibitors of TNF activity (e.g., Enbrel and Remicade) suggest that these drugs may produce certain undesired immunomodulatory effects (e.g., increased incidence of infections). To understand the potential effect of TNF-αinhibition on innate and acquired immunity, a 4-week immunotoxicity study was conducted in female B6C3F1 mice administering anti-murine TNF-αantibody once a week at approximately 250 µg/dose by intraperitoneal (IP) injection. In addition to standard toxicity endpoints (including body weights, clinical observations, and spleen, thymus, liver, and kidney weights), spleen IgM antibody response to a T-dependent antigen, sheep erythrocytes, natural killer (NK) cell activity, and splenic and peripheral blood lymphocyte subsets were evaluated. Cyclophosphamide and Anti-Asialo GM1 (Rabbit) antibody were used as positive controls. No treatment-related effects on body or organ weights were observed.

Anti-TNF Ab treatment resulted in an approximate 35% decrease in IgM AFC response/10^6 splenocytes and an approximate 30% decrease in the absolute numbers of peripheral blood suppressor-cytotoxic T-cells (CD3+CD8+). No treatment-related effects in peripheral blood or splenic total B and T cells, splenic macrophages, or splenic T-cell subsets were noted. Although not statistically significant, NK cell activity was increased when compared with the vehicle control. These results suggest that inhibition of TNF-αactivity may affect the acquired immune response and therefore may contribute to the immunomodulatory effects seen with the inhibitors of TNF-αactivity.

714 DIESEL EXHAUST PARTICLES INCREASE LUNG INJURY AND INFLAMMATION DURING EARLY SENSITIZATION IN BROWN NORWAY RATS.

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Diesel exhaust particles (Department) possess demonstrated adjuvant activity in both human and experimental respiratory allergy, yet the mechanisms of adjuvancy remain poorly described. We have previously reported that intratracheal (IT) instillation of Department prior to allergic sensitization enhances pulmonary allergic responses to house dust mite (HDM) in Brown Norway (BN) rats. The purpose of the present study was to track the adjuvant effects of Department during early sensitization to HDM. BN rats were instilled IT on day 0 with either 100 or 500 μg Department (SRM 2975) or saline only and immunized IT 24 hr later with 10 μg HDM or saline (sham-immunized) on days 1 and 3. Lung injury and inflammatory status were assessed 6 and 24 hr after the final immunization. Department (500 μg) significantly increased total protein, lactate dehydrogenase (LDH) and total inflammatory cell numbers in bronchoalveolar lavage (BAL) fluid in both HDM and saline-treated rats. Specifically, Department (500 μg) induced a sharp increase in the total number of neutrophils in the BAL fluid, peaking at 6 hr and remaining significantly elevated at 24 hr after the final immunization. Department (500 μg) also increased total numbers of eosinophils in the BAL fluid at 24 hr. In order to account for Department-enhanced inflammatory cell influx during early sensitization, we quantitated chemokine levels in BAL fluid and lung homogenate at these time points. The kinetics of chemokine production in response to Department exposure indicated an early increase in MIP-2 and RANTES at 6 hr, followed by a decrease at 24 hr after the final immunization in both HDM-and sham-immunized rats. These results show that Department causes an early increase in lung injury and inflammatory cell influx and a coincident shift in local chemokine production, which may contribute to activation of specific immune responses. (Supported by NCSU/EPA Training Agreement CT826512010 and NIH grant # ES11245-01) (This abstract does not reflect EPA policy.)

715 TIME DEPENDENT INCREASE IN ALVEOLAR MACROPHAGE PHAGOCYTOSIS FOLLOWING INCUBATION WITH CARBON BASED PARTICLES.

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Epidemiological studies have shown that a rise in PM10 is associated with exacerbations of respiratory disease, increases in respiratory infection and increased morbidity. The mechanism of these effects are not well understood, but the ability of the lung to protect and remove inhaled particles will determine the outcome in terms of adverse health effects. We investigated whether exposure to carbon based particles could alter subsequent phagocytic ability. AM, isolated from rats, were exposed *in vitro* for 2, 4 or 24 hours to varying doses of fine carbon black (CB), ultrafine carbon black (UFCB), carbon black coated with 5% copper sulphate (CBCu), carbon black coated with a mixture of 1% iron, copper, vanadium and nickel sulphates (CBMix) and diesel exhaust particles (Department) (1, 10, 25 and 50ug/100, 000 cells). Secondary phagocytosis was assessed over a 3 hour period by monitoring ingestion of FITC labeled E.coli. After 2 hour incubation, all of the particle types increased secondary AM phagocytosis regardless of dose. This increase was sustained following 4 and 24 hours of particle incubation with the CBMix particles, but not with the other particle types which decreased to control levels. Biochemical analysis of supernatants revealed no change in LDH and marginal increases in total protein only after 2 hours incubation. Lysosyme levels were elevated after the 4 hour treatment period with all particles, compared to control levels. TNF alpha protein levels were also elevated 15-20% after 24hr incubation regardless of concentration or type of particle used. The data show that short term incubation with carbon based particles increases secondary AM phagocytosis with subsequent release of bactericidal enzymes and pro-inflammatory cytokines. The results suggest that host defenses may be activated in response to environmental insult. This abstract does not reflect EPA policy.

716 *IN OVO* MODULATION OF T CELL-DEPENDENT IMMUNE FUNCTIONS: POSSIBLE ROUTES OF Pb-INDUCED IMMUNOTOXICITY IN CHICKENS.

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We studied potential thymus-based routes leading to Pb-induced T cell-dependent immunotoxicity after early developmental Pb exposure in the chicken. Using Cornell K strain White Leghorn chickens, the capacity of Pb to directly alter thymocyte function was evaluated with both an in vivo/*ex vivo* system and direct *in vitro* exposure. When the embryo was exposed to Pb (400 μg/egg) at 12 days of embryonic development (E12), the *ex vivo* production of interferon-γ (IFN-γ) by thymocytes collected from four-week old chickens decreased significantly compared to the control. Decreased IFN-γ production was also observed among thymocytes directly exposed to Pb *in vitro* and stimulated with thymic stromal supernatant but not ConA. These results suggest that Pb could directly influence thymocyte function in the absence of the thymic microenvironment. In the reverse experiment, when thymic stromal cells were exposed to Pb *in vitro*, Pb caused no change in the stimulating capacity of thymic stromal cells for thymocyte IFN-γ production. To examine the possible indirect effect of Pb on thymocyte function, thymulin was introduced along with a sublethal dose of Pb into fertilized chicken eggs through the air sac at E12. After hatching, female chickens were immunized with BSA, and then a delayed-type hypersensitivity (DTH) reaction and IFN-γ production were measured. Supplementation of thymulin partially reversed Pb-induced DTH depression without any change in IFN-γ production. Embryonic exposure to thymulin alone did result in a decreased DTH response. These results suggest that thymulin can influence Pb-induced immunotoxicity and raise the question whether endocrine status during Pb exposure might be important. Therefore, it is likely that Pb exposure could alter thymocyte populations directly; but indirect effects, those partially involving endocrine considerations, cannot be excluded.

717 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) DOES NOT DIRECTLY ALTER THE PHENOTYPE OF MATURING B CELLS IN A MURINE CO-CULTURE SYSTEM.

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2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD), acting through the aromatic hydrocarbon receptor (AhR), elicits numerous toxicological effects, including immunosuppression. Previous work from our laboratory has suggested that TCDD exposure in mice is associated with altered lymphopoietic development, in particular altered B cell phenotype in the bone marrow. It remains to be determined which specific hematopoietic populations or sub-populations within the marrow cavity are directly targeted by TCDD. To examine the effects of TCDD on developing B cells *in vitro*, a stromal co-culture model was used. Primary bone marrow cells from 6-7 week old C57Bl/6 mice were co-cultured separately on two AhR-containing stromal cell lines (M2-10B4 and S17) that support B cell development in the presence of IL-7. The co-cultures were treated with 0 to 10 nM TCDD. Shifts in phenotype were quantified by cell surface marker staining and flow cytometry. Four populations in the maturing B cell (very early pre-pro-B, B220-/CD24-/CD43+; pre-pro-B, B220+/CD24-/CD43+; pro-B, B220+/CD24+/CD43-; and pre-B, B220+/CD24+/CD43-) were defined for quantification. The only statistically significant effect of TCDD was within the pre-pro-B population in cultures with the S17 stromal cell line. The increase in number of cells with this phenotype was seen in cultures with both wild type cells and AhR -/- primary bone marrow cells. These results suggest that the maturing B220+ B cell is not the direct target for TCDD-induced bone marrow B cell alterations. Supported in part by National Institutes of Health Grant ES02515, Center Grant ES01247, and Training Grant ES07026.

718 INDUCTION OF INTERLEUKIN-1, TUMOR NECROSIS FACTOR-ALPHA AND TRANSFORMING GROWTH FACTOR-BETA 1 IN ANILINE-INDUCED SPLENIC TOXICITY.

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Exposure to aniline causes selective toxicity to the spleen, leading to a variety of sarcomas, most commonly fibrosarcomas, in rats. Fibrosis appears to be an important preneoplastic event in the aniline-induced splenic toxicity. We have shown that aniline exposure causes oxidative stress in the spleen, which may cause induction of fibrogenic cytokines leading to splenic fibrosis. The present study was undertaken to determine the possible role of cytokines in the fibrogenic response of aniline. Male SD rats were given 0.5 mmol/kg/day aniline hydrochloride through drinking water for 30 days, while control rats received drinking water only. Aniline treatment

caused increases in spleen weight and splenocyte population. The splenocytes from aniline-treated rats showed greater mitogenic responses to concanavalin A and pokeweed mitogen, as detrmined by MTT assay. Interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-alpha) and transforming growth factor-beta 1 (TGF-beta 1) in the culture supernatants of splenocytes (cultured for 24 hr) from control and aniline-treated rats were quantitated using specific ELISA kits. All three cytokines, i.e., IL-1, TNF-alpha and TGF-beta 1 showed increased secretion in the culture supernatants from aniline-treated rats as compared to the controls. Greater induction of IL-1, TNF-alpha and TGF-beta 1 in the splenocytes from aniline-treated rats, alongwith morphological evidence of fibrosis, suggest a role for these cytokines in the aniline-induced splenic fibrosis.

719 PERINATAL EXPOSURE TO ATRAZINE ALTERS JUVENILE IMMUNE FUNCTION IN SPRAGUE-DAWLEY RATS.

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High doses (50-300mg/kg) of the chloro-triazine herbicide atrazine (ATR) alter endocrine function (e.g., luteinizing hormone and triidothyronine secretion) in juvenile or adult rats; however, ATR appears to have no effect on adult immune function. Exposure of nursing dams to ATR produces persistent changes in the immunomodulatory hormone prolactin in their offspring, reflecting the familiar trend of greater neonatal susceptibility. Furthermore, the immunoregulatory properties of prolactin and thyroid hormones suggest that ATR may have perinatal immunomodulatory potential either directly or through transitory hypoprolactinemia or hypothyroidism. Therefore, to evaluate ATR as a developmental immunotoxicant, pregnant Sprague-Dawley rats were subjected to 4 separate treatment regimes from gestational day 10 through post-natal day (PND) 23: 1) ATR (35mg/kg/day) in methylcellulose *via* gavage, 2) bromocryptine (0.2mg/kg/2x/day) *s.c.* to induce hypoprolactinemia, 3) propylthiouracil (2mg/kg/day) *s.c.* to induce hypothyroidism, or 4) methylcellulose via gavage for control dams. Offspring were then tested after PND 50 for: 1) natural killer (NK) cell function, 2) delayed-type hypersensitivity (DTH) response to bovine-serum albumin (BSA), and 3) phagocytosis of latex beads by peritoneal macrophages. Exposure of pregnant dams to ATR decreased NK cell function and DTH responses in male offspring but increased NK function in female offspring. These results demonstrate that perinatal exposure to ATR produced gender specific changes in immune function that persist into adulthood. Although transitory hypothyroidism increased phagocytic response in offspring, neither hypothyroidism or hypoprolactinemia altered NK or DTH responses, suggesting that immune changes associated with ATR are not mediated through suppression of these systems. (This abstract does not reflect EPA policy and was supported in part by the NCSU/EPA Cooperative Training Program in Environmental Sciences Research, Training Agreement CT826512010 with North Carolina State University).

720 ESSENTIAL ROLE OF DOUBLE-STRANDED RNA-ACTIVATED PROTEIN KINASE PKR IN VOMITOXIN-INDUCED MAPK PHOSPHORYLATION, TNF-ALPHA PRODUCTION AND APOPTOSIS IN HUMAN U-937 CELLS.

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Vomitoxin (VT, deoxynivalenol) and other trichothecene mycotoxins inhibit translation and trigger a ribotoxic stress response that results in phosphorylation of mitogen-activated protein kinases (MAPKs) as well as induction of TNF-alpha expression and apoptosis in cloned murine macrophages. Previous studies with the chemical inhibitors 2-aminopurine and adenine have suggested that activation of double-stranded RNA-activated protein kinase (PKR) are involved in these events. The purpose of this study was to employ a stable transformant of human promonocytic U-937 cells containing an antisense PKR expression vector (CUA cells) to evaluate the roles of PKR in mediating VT-induced MAPK activation, translational arrest, TNF-alpha expression, and apoptosis. Significant reduction and delay of VT-induced phosphorylation of MAPKs was observed to occur in a dose-dependent fashion in CUA cells as compared to control cells transfected with vector only (CUC2 cells). Specifically, JNK 1 and JNK 2 phosphorylation was inhibited completely in CUA cells. ERK2 (p42) but not ERK1 phosphorylation was reduced whereas p38 was only partially suppressed. The ID50s for leucine incorporation in cells treated with VT or the translational inhibitors anisiomycin (AN) and emetine (EM) were increased by 25 to 50% in CUA as compared to CUC2. Stimulation index for VT-induced TNF-alpha expression was markedly decreased in CUA as compared to CUC. Both caspase activation and DNA fragmentation following VT, AN and EM exposure were markedly suppressed in CUA as compared to CUC2. The results suggest the PKR may be essential for VT-induction of MAPK phosphorylation and for resultant elevations in cytokine production and apoptosis in leukocytes (Supported by NIEHS Grants ES-09521 and ES-03358)

721 EVIDENCE FOR THE INVOLVEMENT OF THE CB2 RECEPTOR IN THE MODULATION OF INTRACELLULAR CALCIUM ($[Ca^{2+}]_i$) BY Δ^9-TETRAHYDROCANNABINOL (Δ^9-THC) IN HUMAN T CELLS.

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Cannabinoids are a class of over 60 structurally-related plant-derived compounds present in the marijuana plant, *Cannabis sativa.* Cannabinoids are potent inhibitors of IL-2 production in T cells. In the present studies, HPB-ALL and Jurkat E6-1 human T cell lines were employed to elucidate the mechanism of IL-2 inhibition by Δ^9-THC, the primary psychotropic cannabinoid in marijuana. Previous work from this laboratory has demonstrated by Northern analysis that both cell lines lack transcripts for the central cannabinoid receptor, CB1; and that the transcript for peripheral cannabinoid receptor, CB2, was normal sized (2.4Kb) in HPB-ALL cells, but aberrant in Jurkat E6-1 cells (2.3, 4.5 and 5.7Kb). The objective of the present studies was to examine the effect of Δ^9-THC on $[Ca^{2+}]_i$ and inhibition of phorbol ester (PMA) and ionomycin (Io) induced IL-2 production in HPB-ALL and Jurkat E6-1 cells. Δ^9-THC (10μM) induced a rapid and transient rise (500-700nM) in $[Ca^{2+}]_i$ in HPB-ALL cells as compared to a slow and modest rise (80-100nM) in Jurkat E6-1 cells. This rise in $[Ca^{2+}]_i$ was attenuated in presence of the CB2 receptor antagonist, SR144528 (1μM), in HPB-ALL cells, but not in Jurkat E6-1 cells. In addition, Δ^9-THC inhibited IL-2 secretion in HPB-ALL cells in a concentration dependent manner, but not in Jurkat E6-1 cells. These data suggest that Δ^9-THC acts to disrupt $[Ca^{2+}]_i$ regulation in a CB2 receptor-dependent manner which may contribute to the inhibition of IL-2 secretion. (Supported in part by NIH grant DA07908).

722 PARADOXICAL MODULATION OF T CELL GROWTH BY THE ENDOGENOUS CANNABINOID, 2-ARACHIDONYL GLYCEROL, CAUSES AN INHIBITION OF THE T CELL GROWTH FACTOR, IL-2, AND A CONCURRENT ENHANCEMENT OF T CELL PROLIFERATION.

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The putative endogenous cannabinoid, 2-arachidonyl glycerol (2-AG), is an arachidonic acid derivative that has been shown through radioligand binding studies, to bind with affinity to the cannabinoid receptors, CB1 and CB2. 2-AG mimics some of the immunomodulatory effects of the plant-derived cannabinoids, Δ^9-THC and cannabinol, including inhibition of cytokine production. The first objective of the present studies was to determine the role of the CB1 and CB2 receptors in 2-AG-mediated inhibition of IL-2. In activated murine splenic and thymic T cells, 2-AG causes a marked and concentration-responsive inhibition of IL-2 that is more potent than anandamide, another putative endogenous cannabinoid. The CB1 and CB2 antagonists, SR141716A and SR144528 respectively, did not antagonize the inhibition of IL-2 mediated by 2-AG or anandamide. The second objective of the present studies was to determine the effect of 2-AG upon T cell proliferation. Interestingly, 2-AG causes a concurrent concentration-responsive enhancement of T cell proliferation at the same concentrations at which it inhibits the production of IL-2. 2-AG caused a significant enhancement of T cell proliferation at 1 μM and an approximate 50% increase in proliferation at 10 μM. Neither anandamide nor arachidonic acid caused an enhancement of T cell proliferation. These data suggest that 2-AG enhances T cell proliferation while paradoxically and concurrently inhibiting the production of the T cell growth factor, IL-2, in activated murine splenic and thymic T cells. These data also suggest that the 2-AG-mediated enhancement of T cell proliferation is unique to 2-AG and not common to arachidonic acid and its derivatives. (Supported in part by NIH Grant DA 12740)

723 CONCENTRATION-DEPENDENT BIFUNCTIONAL EFFECTS OF TGF-β_1 on Immunoglobulin Production: Role for Smad3 in IgA Production *in vitro.*

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TGF-β_1 is a critical regulator of humoral and cell-mediated immune homeostasis. The molecular mechanisms responsible for the regulation of immune cell effector function by TGF-β_1 are not well defined. We demonstrate bifunctional augmenta-

tion and inhibition of *in vitro* T cell-dependent anti-SRBC- and T cell-independent DNP-Ficoll-induced AFC responses by femtomolar and picomolar concentrations of TGF-β_1, respectively. To investigate the molecular mechanisms responsible for the inhibitory effects of TGF-β_1 on T cell-dependent and -independent AFC responses, splenocytes were obtained from mice homologous for a null mutation in the gene encoding the TGF-β receptor-activated Smad3 ($Smad3^{Exon8-/-}$) and sensitized *in vitro* to either anti-SRBC or LPS. Inhibition by TGF-β_1 was markedly reduced in the $Smad3^{Exon8-/-}$ B cells compared with age-matched wild type littermates ($Smad3^{+/+}$). Moreover, LPS-sensitized $Smad3^{Exon8-/-}$ B cells were less sensitive to TGF-β_1-induced inhibition of IgM secretion as determined by ELISA. A role for Smad3 in IgA production *in vitro* was also investigated. TGF-β_1 did not induce IgA production in LPS-sensitized $Smad3^{Exon8-/-}$ B cells under conditions that yielded significantly increased IgA production $Smad3^{+/+}$ B cells. In conclusion, our results demonstrate a bifunctional, concentration-dependent effect of TGF-β_1 on Ig production. These results are also provide evidence for a role by Smad3 in IgA production by TGF-β_1 *in vitro*.

724 ENHANCEMENT OF TNF-α mRNA STABILITY BY DEOXYNIVALENOL THROUGH ACTIVATION OF P38 KINASE.

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Deoxynivalenol (vomitoxin, VT), a common fungal metabolite in grains, up-regulates proinflammatory cytokine gene expression. This up-regulation is controlled by both transcriptional and post-transcriptional mechanisms. We have recently shown that VT up-regulates TNF-α gene expression post-transcriptionally *via* enhanced mRNA stability. Since stabilization of cytokine mRNA is often related to activation of mitogen activated protein kinases (MAPKs), we investigated the role of these kinases in enhanced TNF-α mRNA stability in RAW 264.7 macrophage cells. p38 kinase, ERK1, and ERK2 were found to be activated by VT. The effects of VT on increased mRNA stability in the presence of MAPK inhibitors were studied in lipopolysaccharide (LPS)-stimulated cells. After 4 hr stimulation by LPS, a transcription inhibitor, 5, 6-dichloro-1-beta-D-ribofuranosyl benzimidazole (DRB), with or without MAPK inhibitor (SB203580 or PD98059) was added to culture and VT-mediated changes in TNF-α mRNA stability were measured using a ribonuclease protection assay (RPA). Increased TNF-α mRNA stability by VT was abrogated in the presence of p38 kinase inhibitor (SB203580). Treatment of ERK inhibitor (PD98059) did not affect stabilization of TNF-α mRNA by VT. These results suggest that VT up-regulates TNF-α gene expression by increasing mRNA stability *via* activation of p38 kinase.

725 EXTRACELLULAR METALLOTHIONEIN ALTERS THE T_H RESPONSE TO COGNATE ANTIGEN PRESENTED BY ANTIGEN PRESENTING CELL.

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Metallothionein(MT) is a stress-induced protein that can modulate several key immune mechanisms. In previous work, MT has been demonstrated to decrease *in vivo* and *in vitro* measures of humoral and cell-mediated immunity. MT has also been shown to bind to leukocyte plasma membranes both *in vivo* and *in vitro*. These observations suggest that MT may alter cell function through interaction with surface proteins preventing effective cell:cell interaction. We have used antigen presenting and cell proliferation assays to address this possibility. Our results show that MT can significantly interfere with *in vitro* antigen recognition by T cells co-cultured with antigen presenting cells (APCs). In these experiments, MT decreased OVA-stimulated DO-11.10 T cell cytokine secretion by 20-30% when compared to vehicle control. This MT-mediated suppression of T cell responses to antigen also occurred in MT-null T cells when exogenous MT was added to cultures, indicating that the induction of intracellular MT is not required for the mechanism of suppression. Furthermore, using an array of H-2s restricted T cell lines that are each specific for different OVA epitopes, we observed MT-mediated suppression of IL-2 and IL-4 cytokine secretion in a majority of clonal T cell responses to antigen presented by APCs. Intriguingly, there was one OVA-specific T cell clonal response that was enhanced by the presence of MT. DO-11.10 T cell responses were also suppressed in the presence of MT when stimulated by fixed APCs, suggesting that MT need not be present during antigen uptake and processing to suppress subsequent T cell responses. T cell proliferation that is experimentally induced with anti-CD3 antibodies is also suppressed by the presence of MT, suggesting that MT may interfere with effective T cell responses through the T cell receptor:MHC immune synapse. These observations further extend the idea that metallothionein synthesized as a consequence of cellular stress may play an important role in the onset of stress-induced immune dysfunction.

726 SODIUM METHYLDITHIOCARBAMATE SUPPRESSES INTERLEUKIN-2, INTERLEUKIN-4, AND INTERFERON-γ PRODUCTION AFTER STIMULATION IN B6C3F1 MICE.

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Sodium methyldithiocarbamate (SMD) is a commonly used fungicide, nematocide, and herbicide in agricultural soils, which is immunotoxic in mice. Since other dithiocarbamates (pyrrolidine dithiocarbamate, e.g.) have been implicated in cytokine alterations in mice, we investigated the effect of SMD on cytokine production. When pretreated with SMD (300 mg/kg) and then stimulated with anti-CD3 (3 μg/mouse), B6C3F1 mice showed marked suppression of serum cytokine production at 90 minutes post-stimulation. SMD suppressed Interleukin-2 (IL-2) (1323.2 pg/ml) compared to anti-CD3 alone (4653.2 pg/ml). Similar SMD-induced suppressions were seen with IL-4 (433.2 compared to 1091 pg/ml), and Interferon-γ (IFN-γ) (1398.0 compared to 5361.8 pg/ml). Expression of IL-2, IL-4, IL-13, and IFN-γ mRNA was significantly suppressed by SMD at 45 minutes, but the cytokines were not significantly different at 90 minutes post-stimulation when evaluated by RNAse protection assays. Since SMD increases serum corticosterone by inducing a stress response, aminoglutethimide was administered to evaluate the role of glucocorticoids in cytokine suppression. Although the overall levels of cytokines were increased (possibly due to the lack of corticosterone to regulate the cytokine expression), a similar pattern of cytokine suppression was noted when SMD was administered in the absence of corticosterone. Therefore, it is unlikely that corticosterone plays a role in SMD-induced cytokine suppression. Since other dithiocarbamates suppress cytokines by blocking NF-κB signaling, we evaluated the effect of a single dose of SMD (300 mg/kg) over a time course. SMD depleted basal levels of NF-κB in the nucleus of splenic T-cells by 50% at 60 minutes post-administration. NF-κB levels then increased to normal by 80-120 minutes after administration of SMD. Therefore it is possible that SMD suppresses cytokine expression by blocking NF-κB signaling in the cell. This work was supported by NIH grant #ES09158.

727 LOW CONCENTRATIONS OF INORGANIC MERCURY INHIBIT RAS ACTIVATION DURING T CELL RECEPTOR MEDIATED SIGNAL TRANSDUCTION.

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Mercury is widespread in the environment, and consequently there are large populations who are currently exposed to low levels of mercury as a result of ubiquitous environmental factors. Whether these environmental levels of mercury are harmful is a matter of current debate, with epidemiological and animal studies suggesting detrimental effects on the immune and nervous systems. However specific cellular effects of low concentrations of mercury have been difficult to characterize. We now demonstrate that sub-toxic concentrations of $HgCl_2$ can potently (maximal at 1 μM) increase Ras.GTP levels in Jurkat, a human T cell line. Remarkably, this activation of Ras occurs without a concomitant increase in MAP kinase activation, suggesting that mercury may direct Ras into a non-productive state. In addition to its direct effect on Ras, concentrations of $HgCl_2$ as low as 0.6 μM inhibited the ability of the T Cell Receptor to activate Ras and MAP kinase. The inhibitory effect of mercury is selective, as activation of MAP kinase by phorbol diesters remain intact. Since the Ras/MAP kinase pathway is both highly conserved, and central to signal transduction processes mediated by a myriad of diverse membrane receptor systems in a variety of cell types, these results suggest a mechanism for adverse health effects resulting from exposure to low levels of mercury. They also support a model for regulation of the Ras/MAP kinase pathway, whereby partial but unproductive activation of Ras can diminish signaling from cell surface receptors.

728 MECHANISMS OF PPARγ-MEDIATED PRE-B CELL APOPTOSIS.

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The role of PPARγ in adipocyte differentiation and function is well recognized. In addition, PPARγ is expressed strongly in the immune system and also may influence immune cell function. As shown by propidium iodide staining, DNA ladder formation, and PARP cleavage, PPARγ agonists (15-deoxy-$\Delta^{12,14}$-prostaglandin J2, ciglitazone, troglitazone and rosiglitazone) rapidly (6 hours) induced apoptosis in pro/preB cells (BU-11), immature B cells (WEHI-231) and plasma cells (HS Sultan, U266, IM9). We hypothesize that activation of PPARγ and its dimerization partner retinoid X receptor (RXR) induces B cell apoptosis. Untreated B cells express PPARγ only in the cytoplasm. Following treatment with ciglitazone, the receptor translocates to the nucleus, binds a consensus PPARγ -binding nucleotide

sequence derived from the acetyl CoA oxidase promoter, and initiates transcription, as indicated by western blot, EMSA, and increased PPARE-driven reporter activity. The role of PPARγ in apoptosis is supported further by the fact that co-treatment with 9-cis retinoic acid (a specific agonist for RXR) synergistically increased the incidence of apoptotic B cells. Given previous studies indicating that PAH-induced pre-B cell apoptosis is triggered by NF-κB down-regulation, we also hypothesized that alteration of NF-κB-DNA binding is involved in PPARγ agonist-induced apoptosis. Ciglitazone caused a 3.6-fold increase in NF-κB binding to the κB site in the upstream regulatory element of c-myc. NF-κB-DNA binding increased as early as 1 hour post-treatment and was maximal 6-7 hours post-treatment; the p50, p65 and c-rel subunits of NF-κ B all contributed to this increase. In parallel with the apoptosis dose-response curves, co-treatment with 9-cis retinoic acid and ciglitazone decreased the ciglitazone dose required to activate NF-κB, suggesting that PPARγ-RXR heterodimer activation results in NF-κB activation. In summary, activation of PPARγ appears to initiate apoptotic signaling in B cells of multiple maturation stages, potentially through the activation of NF-κB.

729 CUMULATIVE IRRITATION (21 DAY) STUDY OF POLYVINYLIDENE FLUORIDE COATINGS ON HUMAN SUBJECTS.

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Introduction: Our laboratory has developed a unique formulation, EpikieaTM coatings, for direct application to skin. This blend of polyvinylidene fluoride (PVDF) and acrylic polymers, is safe and remains intact on human skin for extended periods of time, making it beneficial in the prevention of irritation due to incontinence. Methods: PVDF coatings were applied in a randomized, cross-over design to the backs of 26 human subjects over a 21 day period and scored for irritation. Subjects were 21 to 75 years of age, had no clinically significant history of skin, kidney, lung disease, or diabetes and no evidence of any skin lesions. Subjects served as their own controls. Each subject had 10 sites of 4x4 cm2 areas on their mid-back, beneath the angles of the scapulae, demarcated with an indelible skin marker. Subjects reported for treatment and evaluation daily during weekdays. No treatment or evaluation took place over weekends. During daily treatment, coatings were removed, skin was scored for irritation, and the coatings were re-applied. Daily irritation scoring was made 24 hours after each application of the PVDF coating test material (except weekends). After scoring, the treatment area was cleaned with soap and water and air dried, and coatings were reapplied to the same sites. Visual scoring was made for erythema/eschar (0 - no erythema to 4 - severe erythema/ slight eschar formation) and edema (0 - no edema to 4 - severe edema/ raised more than 1 mm). In addition, non- invasive testing at each test site was performed using three instruments: a colorimeter which measures the color of the skin and erythema; an evaporimeter which measures the moisture content of the skin; and a laser-Doppler, which measures the microcirculatory blood flow. Results: No evidence of any erythema or edema was found on any subject at any time. There was never a score greater than 0 on any site during the 21 day study. Finally, none of the instruments showed any adverse effects. Conclusion: PVDF coatings are non-irritating to humans.

730 IMMUNOMODULATION FOLLOWING ORAL ADMINISTRATION OF THE ANTIFUNGAL AGENT SCH 56592.

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SCH 56592 is a broad-spectrum azole antifungal drug in development for treatment of mucosal and systemic fungal infections. Immunocompetence of CD-1 mice following daily dietary dosing of 10, 30 and 90 mg/kg SCH 56592 for 1 and 3 months was assessed. A 1-month recovery period following 3 months of dosing was also included. Immunotoxicity assessment included lymphoid organ weights and cellularities, hematology, leukocyte phenotype analysis of blood and spleen, induction of antibody-forming cells (AFC), and natural killer (NK) cell activity. Animals dosed with SCH 56592 had an increased incidence of a common background ear lesion in CD-1 mice, but no changes in body weight or food consumption. Dose-related increases in spleen, liver and adrenal weights, and in spleen cellularity occurred; thymus weight and cellularity were unaffected. There were mild to moderate increases in leukocytes due to increases in monocytes, neutrophils and/or lymphocytes at 30 and 90 mg/kg after 1 and 3 months of dosing; these increases were still apparent 1 month postdose at 90 mg/kg. There was a dose-related trend toward increased NK cell number and monocyte number and percentage; these changes resolved following recovery. Splenic lymphocytes were also increased. SCH 56592 had no effect on AFC production in males; females were unaffected at 1 month, but at 3 months they exhibited a statistically significant increase in AFC at 10 mg/kg, and a statistically significant decrease in AFC at 90 mg/kg. Changes were minimal, and resolved following recovery. NK activity was increased in both sexes at both 30 and 90 mg/kg following a 1-month administration, and in males only following 3 months of dosing. Following recovery, NK activity returned to control levels in all mice. These data suggest that administration of SCH 56592 is associated with a modest immunostimulation in CD-1 mice. The resolution of most changes following cessation of dosing suggests that SCH 56592 has no permanent effect on the functional integrity of the immune system.

731 ASSESSMENT OF IMMUNOMODULATORY POTENTIAL OF FTY720 IN A MURINE CONTACT ALLERGY MODEL.

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FTY720 is a chemically modified fungal product, which is in development for the use in organ transplantation to prevent allograft rejection. Contact allergy (CA) was induced by a 3 day topical treatment with dinitrochlorobenzene (DNCB) on the shaved back of BALB/c mice. 12 days later, elicitation of CA was provoked by a 3 day topical application of DNCB on the dorsum of both ears. FTY720 was administered orally during the elicitation phase in sensitised mice and the concurrent non-sensitised control animals. Necropsy was performed one day after the last treatment. In a preceding experiment FTY720 caused a marked reduction of peripheral lymphocyte counts and a marked decrease of ear-draining lymph node (LN) weights at 0.1 and 1mg/kg, when applied for 3 days together with topical application of DNCB. Oral application of 0.1 and 1 mg/kg FTY720 during elicitation of CA led to a decrement in the DNCB-caused hyperplasia of ear-draining LN and a reduction in the allergy-relevant increase in ear weight. However, evaluation of IL-2 receptor expression on the surface of CD4 T cells and the generation of CD4 T cell blasts by flow cytometry of cells from the ear-draining LN did not reveal significant changes in comparison to the respective control. In contrast, the immunosuppressant FK506 caused a marked reduction in this study not only of the LN hyperplasia and allergy-relevant ear swelling, but also of IL-2R expression and CD4 T cell blastocytosis. In addition, the cytokines IL-2, IL-4 and IFN gamma were determined in supernatants of restimulated LN cells derived from FTY720 and DNCB-treated mice. No substantial changes caused by FTY720 in the DNCB-induced pattern of these cytokines were observed. The presented findings correspond with the mechanism of action of FTY720, which prevents immune-mediated rejection of allotransplants by distracting lymphocytes from the periphery rather then by suppressing their activation. This study shows that a contact allergy-based murine model can be applied to investigate the influence of test articles on the immune systems function.

732 GENISTEIN MODULATES NK CELL ACTIVITY, ANTI-CD3-MEDIATED T CELL PROLIFERATION AND PHENOTYPIC MARKER EXPRESSION BY THYMOCYTES AND SPLENOCYTES IN F_0 AND F_1 GENERATIONS OF C57BL/6 MICE.

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Although the phytoestrogen genistein (GEN) has been hypothesized to have beneficial health effects in adults, concern exists about the long-term adverse effects of this compound. The objective of the present study was to evaluate the effect of GEN on the immune system in adult and developing mice. Pregnant C57BL/6 mice were exposed to GEN at low (L: 25 ppm), middle (M: 250 ppm) and high (H: 1250 ppm) concentrations in feed starting on day 14 of gestation. The offspring were exposed to GEN gestationally and lactationally, and to GEN-containing feed after weaning on postnatal day 21. Immunological evaluation was performed on postnatal day 42. In dams, exposure to GEN increased terminal body weight (M and H), splenic T cell and NK cell numbers (M), and the activity of NK cells (M). In F1 males, GEN increased terminal body and spleen weights (L and M), the number of $CD4^+CD8^+$ and $CD4^-CD8^-$ thymocytes (L), and the number of splenic T-cell subsets and NK cells (L and M). Moreover, splenic NK cell activity and anti-CD3-mediated splenocyte proliferation were increased in all dose groups. In F1 females, the percentages of $CD4^-CD8^+$ and $CD4^-CD8^-$ thymocytes (L and M), and the splenic $CD4^+CD8^-$ and $CD4^+CD8^+$ T cells (L and M) were increased. In contrast, the percentage and number of $CD4^+CD8^+$ thymocytes were decreased in the middle dose group. Exposure to GEN decreased the percentages of splenic NK cells in all dose groups, while decreased the activity of splenic NK cells in the low dose group. Overall, the results demonstrate that GEN can modulate the immune system with greater effects occurring in developing mice. The sexual dimorphic effects of GEN on the immune responses of F_1 male and female mice suggest that GEN may function as an endocrine disrupter. (Supported in part by the Jeffress Memorial Trust and NIEHS Contract ES 55387).

733 STRAIN-DEPENDENT PRIMARY AND SECONDARY DICLOFENAC-INDUCED POPLITEAL LYMPH NODE REACTIONS.

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Diclofenac (DF) is associated with immune-mediated drug hypersensitivity reactions (IDHRs). Although the mechanisms causing DF-induced IDHRs remain to be defined, it is thought that IDHRs are controlled by genetic factors. The aim of this study was to examine the immunomodulating potential of DF in two genetically diverse and MHC-mismatched mouse strains (BALB/c, H2d and B6, H2b). This study used a popliteal lymph node assay (PLNA) that was conducted in naïve and orally pretreated mice (3 weeks after a single dose of DF). In naïve mice, DF induced a PLN reaction in both strains that resulted in the accumulation of activated T cells in the PLN. Despite these similarities, strain-dependent effects were observed. For example, there was an increase in the % of CD3+CD4+, and CD3+CD8+ lymphocytes in B6 mice, whereas no increase was observed in BALB/c mice. Oral pretreatment with DF also had a strain-dependent effect. For example, BALB/c mice orally pretreated with DF were rendered hyper-responsive to footpad injection with DF; a significant increase in footpad swelling 24 hours post-injection and significant increases in PLN size, weight and cellularity on day 5 post-injection. By contrast, oral pretreatment with DF had no observable effect on PLN reactions in B6 mice or in congenic BALB/c mice where H2d MHC was replaced with H2b. Furthermore, the hyper-responsiveness in wild-type BALB/c mice was associated with a significant increase in the % of CD8+IL-2+ cells in the PLN. Finally, in *ex vivo* studies, PLN cells isolated from BALB/c mice injected with DF induced proliferation of splenocytes isolated from BALB/c mice orally treated with DF. Collectively, these results suggest that DF has strain-dependent immunomodulating properties in mice. Specifically, BALB/c mice, but not B6, orally pretreated with DF are hyper-responsive to the immunostimulating effects of DF when challenged in the footpad, which is a response that may be mediated, in part, by sensitized CD8+ T cells reacting to DF-modified antigens in the footpad presented by H2d molecules.

734 EVALUATION OF A SURROGATE ANTIBODY FOR SAFETY TESTING. PHARMACOLOGY OF A MURINE ANTI-CD11A ANTIBODY IN MICE.

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Surrogate antibodies are a potential solution to the limited safety testing possible with humanized monoclonal antibodies of restricted species cross reactivity. Prior to use, potential surrogates should undergo rigorous evaluation to assess pharmacological activities in comparison to the clinical agent. The current studies evaluated the pharmacology of an anti-mouse CD11a monoclonal antibody (muM17) as a potential surrogate for efalizumab, a humanized anti-CD11a antibody in development for treatment of psoriasis. CD11a is a subunit of lymphocyte function associated antigen-1, an integrin involved in cell-cell interactions important to leukocyte adhesion and activation. *in vitro* studies examined the binding of muM17 to whole mouse blood and the inhibitory activity of muM17 in a mixed lymphocyte response (MLR) assay. As anticipated muM17 bound to mouse blood cells, with a binding affinity of 2.7 nM. In addition, muM17 had a dose dependent inhibitory effect on the MLR assay with an IC_{50} of 0.1-0.3 ug/ml. In an *in vivo* delayed-type hypersensitivity study in female CD-1 mice, muM17 (3 - 30 mg/kg) was administered subcutaneously on Days 1 and 7. Mice were sensitized and challenged with oxazolone by application to the abdomen and subsequently the ear on Days 3 and 9, respectively. In some animals muM17 was administered only prior to challenge. Ear thickness was measured up to 72 hours after challenge and compared to the unchallenged ear on the same animal. muM17 caused a dose dependent reduction in the delayed type hypersensitivity response up to a maximum inhibition of 100% (≥10 mg/kg). Administration of 30 mg/kg only prior to challenge was still active, but reduced the inhibition to 70% suggesting that the full effect of muM17 involves both inhibition of T cell activation during sensitization as well as trafficking into the tissues upon rechallenge. These studies demonstrate that muM17 has similar pharmacological activities to efalizumab and is active at doses intended for toxicology studies.

735 EVALUATION OF A SURROGATE ANTIBODY FOR SAFETY TESTING. TOXICOLOGY OF A MURINE ANTI-CD11A ANTIBODY IN MICE.

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The development of surrogate antibodies is a potential solution to the limited safety testing possible with humanized monoclonal antibodies with restricted species crossreactivity. Prior to use, potential surrogates should undergo rigorous evaluation to assess pharmacological and toxicological activities in comparison to the clinical agent, as well as to provide dose selection information for subsequent GLP toxicology studies. The current studies evaluated the toxicity of a chimeric mouse/rat IgG anti-mouse CD11a monoclonal antibody (muM17) as a potential surrogate for efalizumab, a humanized anti-CD11a antibody in development for treatment of psoriasis. CD11a is a subunit of lymphocyte function associated antigen-1, a surface molecule involved in cell-cell interactions important to leukocyte adhesion and activation. Female CD1 mice were administered muM17 (0.1 to 30 mg/kg) *via* subcutaneous (SC) injections once a week for four weeks. Clinical observations, body weight, clinical pathology, T cell CD11a expression, immunogenicity and lymphoid organ histopathology were evaluated. Doses of ≥ 3 mg/kg muM17 caused a maximal downmodulation of CD11a on T cells (approximately 90% of baseline values) which was reversible, time to recovery of CD11a expression was dose dependent. muM17 caused a transient increase in peripheral white blood cell counts (2 -3 fold *versus* vehicle treated mice) as a result of increases in lymphocytes and neutrophils. muM17 was immunogenic, but to a greatly reduced extent than the parent antibody M17 (rat anti-mouse CD11a), with an incidence of anti-muM17 antibody positive mice of 1 in 181. There was no histologic evidence of muM17 adverse effects on the spleen or thymus including no lymphocyte depletion. In conclusion, there were no significant toxicities associated with muM17 treatment, downmodulation of CD11a expression and white blood cell changes represented pharmacological activities of the drug consistent with efalizumab.

736 APPLICATION OF A SURROGATE ANTIBODY FOR SAFETY TESTING. DEVELOPMENTAL TOXICITY STUDY OF A MURINE ANTI-CD11A ANTIBODY IN MICE.

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Surrogate antibodies are a potential solution to the limited safety testing possible with humanized monoclonal antibodies with restricted species crossreactivity. The purpose of this study was to detect potential adverse effects of a chimeric mouse/rat IgG anti-mouse CD11a monoclonal antibody (muM17) on CD-1 pregnant female mice and development of the embryo and fetus consequent to exposure of the dam through organogenesis. CD11a is a subunit of lymphocyte function associated antigen-1, a leukocyte surface molecule involved in cell-cell interactions important to leukocyte adhesion and activation. MuM17 is a surrogate antibody for efalizumab, a humanized anti-CD11a antibody in development for the treatment of psoriasis. Previous studies designed to evaluate the surrogate established pharmacological activity of muM17 at doses ≥ 3mg/kg. Further, pharmacodynamic comparisons to efalizumab in terms of downmodulation of CD11a expression on T cells indicated that 3 mg/kg muM17 was an appropriate clinically equivalent dose level and was the lowest dose level selected in this developmental study. Female CD1 mice were administered muM17 (3, 10 or 30 mg/kg) *via* subcutaneous (SC) injections once a week beginning on Day 2 of presumed gestation until Day 16. Exposure to muM17 and the development of anti-muM17 antibodies were determined. Upon caesarian-sectioning on day 18 of presumed gestation the number and distribution of corpora lutea, live and dead fetuses and resorptions and fetal observations were evaluated. Toxicokinetics confirmed exposure to muM17 and there were no detectable antibodies to muM17. There were no muM17 treatment-related effects on the examined parameters including no soft tissue or skeletal malformations. In conclusion, administration of muM17 to pregnant mice was not associated with any adverse developmental effects.

737 IMMUNOGENICITY OF INHALED MURINE CPG OLIGONUCLEOTIDES AS A MUCOSAL ADJUVANT IN COMBINATION WITH RECOMBINANT SEB VACCINE.

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Staphylococcal enterotoxin B (SEB) is an exotoxin derived from the bacterium *Staphylococcus aureus*. SEB, classified as a superantigen and a biological threat agent, is a known inducer of T-cells and can cause illness and death in humans. A USAMRIID-developed recombinant SEB vaccine (rSEBv) protected mice and primates against aerosolized SEB when the vaccine was administered with alhydrogel as an intramuscular (IM) injection. We tested whether administering rSEBv by inhalation would induce mucosal immunity, thus protecting against lethality while also blocking the enteric effects of SEB intoxication. A murine CpG-containing oligonucleotide (CpG) was used as a mucosal adjuvant in conjunction with rSEBv. Mice were dosed with either rSEBv + CpG (5 µg each), CpG alone (5 µg), or saline (sham) by inhalation or 20 µg rSEBv adsorbed onto alhydrogel IM in a prime/three boost vaccination schedule. The use of CpG either alone or with rSEBv induced a transient neutrophilic influx three hours post vaccination with no resulting notable

pathologic sequelae. Immune response was evident in the rSEBv + CpG group characterized by increased serum anti-SEB IgG and IgA in lavage. Pulmonary gene expression profile of mRNA species determined by cDNA array analysis indicated 67 upregulated and six downregulated genes in CpG-treated groups. Analysis of splenic lymphocytes had six times the CD4+ T-cells in the rSEBv + CpG group compared to the rSEBv or sham groups. All groups were then challenged with SEB by aerosol. Survival rates in mice vaccinated by inhalation (92%) were comparable to the mice who were injected (90%) whereas all sham-vaccinated animals died (0%). The results of this study suggest that CpG has the potential to be used as a safe and immunostimulatory mucosal adjuvant when delivered by inhalation.

738 CHARACTERIZATION OF A POTENTIAL ANIMAL MODEL OF AN IDIOSYNCRATIC DRUG REACTION: NEVIRAPINE-INDUCED SKIN RASH IN THE RAT.

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RATIONALE: Idiosyncratic drug reactions are difficult to study in humans due to their unpredictability. Unfortunately, this characteristic also hinders the development of animal models needed for mechanistic studies. Nevirapine (NVP), used to treat AIDS, results in a severe idiosyncratic skin rash in some HIV patients. We found that NVP can also cause a significant rash in some strains of rats. METHODS: Female rats, Lewis (n=6), Brown Norway (BN; n=12), Sprague-Dawley (SD; n=26) and male rats, BN (n=2), were administered NVP, 100-150 mg/kg/day, in their diet. Animals were monitored for development of skin rash, food intake and body weight. At either week 8 of treatment or after development of rash, animals were sacrificed and blood and skin samples acquired. Some animals presenting with skin rash were removed from drug, allowed to recuperate and then rechallenged. To determine the effects of lower doses of NVP female BN rats were treated with 50-75 mg/kg/day (n=4) or 25-40 mg/kg/day (n=4). RESULTS: NVP treatment induced skin rash in 7/26 female SD rats after 4-6 weeks of dosing and in all female BN rats after 3 weeks of dosing; however, male BN rats, female Lewis rats, and female BN rats treated with lower doses of NVP, were not affected. The skin rash consisted of scab-like lesions primarily on the back. Light microscopy revealed a mixed inflammatory infiltrate. Hematology and chemistry tests showed no consistent changes in treated animals. Animals rechallenged with NVP developed skin symptoms faster on reexposure, but the syndrome was different. Instead of presenting primarily with skin lesions, the animals exhibited excessive hair loss commencing at approximately 48 hours post reexposure, occasional skin lesions and general malaise that necessitated sacrifice after less than 2 weeks of treatment. By light microscopy the hair loss was characterized by a perifollicular inflammatory infiltrate. CONCLUSIONS: NVP-induced skin rash in rats may represent a new animal model for the study of drug-induced idiosyncratic skin reactions.

739 ENVIRONMENTAL FACTORS ASSOCIATED WITH INFANT MORTALITY IN SELECTED VIRGINIA REGIONS.

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Socio-economic status has been generally accepted as a primary predictor of susceptibility to Sudden Infant Death Syndrome (SIDS). However; recent evidence suggests that other more influential factors may be associated with SIDS, in particular and infant mortality (IM), in general. A computerized data file of matched birth and death tapes, to one year of age (1995-1998) for District 19 Virginia was obtained from the Division of Vital Records, Virginia Department of Health. The fetal deaths were matched for address and zip code at death in addition to birth weight, race and underlying causes of death. The International Classification of Diseases 9th Revision Clinical Modification (ICD 9 CM) was used to numerically code causes of death. The deaths of eighty-seven (87) infants between 1995 and 1998 in District 19 (a given geographical area of Virginia) were examined to determine if any relationship existed for infant death and selected environmental conditions. Fifty-six (56) of these infants were black and twenty-nine (29) were white (64.4% and 33.3% respectively). The majority of these deaths (40.2%) occurred in one geographic area in Petersburg area code 23803 followed by the area Hopewell City, Emporia and Petersburg 23805 (16.1%, 12.6% and 9.2% respectively). The majority of the Petersburg deaths (76%) were black infants. The suspected leading causes of death in this study were due to congenital anomalies (49%) followed by cardio-respiratory conditions including SIDS (26%) and low birth-weight (14%). All other conditions, as coded by ICD 9 categories, accounted for less than 3% of the deaths. Geographical imaging analysis of District 19 revealed that several neurotoxins were prevalent in areas where IM were highest. Other Districts in Virginia, where IM is relatively high, show similar results. As such, there may be a need to re-examine the risk factors associated with IM particularly among African American women and others disproportionately exposed to select environmental conditions. Supported by UNCF/EPA/PEJER VUU 2001

740 PRIORITIZATION OF TOXIC AIR CONTAMINANTS UNDER THE CALIFORNIA CHILDREN'S ENVIRONMENTAL HEALTH PROTECTION ACT.

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California's legislature enacted the Children's Environmental Health Protection Act requiring Cal/EPA to specifically consider infants and children in setting criteria for toxic air contaminants (TAC) including cancer potency factors and reference exposure levels. The law required the Agency to consider exposure patterns of infants and children, special susceptibility, effects of exposure to more than one TAC with a common mechanism of action, and interactions of TAC and criteria air pollutants in assessing risks to children. The law also requires re-evaluation of the criteria for toxic air contaminants over the next several years to ensure adequate protection of infants and children, and establishment of an initial list of up to 5 TAC that may disproportionately impact children by July 1, 2001. Cal/EPA prioritized the TAC based on known toxicity and exposure. We conducted a focused literature review on 37 TAC looking for specific information pertinent to infants and children's exposure and potential differential response relative to adults. We focused on chemicals associated with developmental, neuro-, respiratory, endocrine, or immune toxicity, and endocrine disruptors. We considered asthma induction and exacerbation as disproportionately impacting children. We considered whether existing criteria were adequate to protect infants and children. After public and peer review by a UC Panel, five TAC were chosen to constitute the initial list: acrolein, polychlorinated dibenzo-*p*-dioxins and dibenzofurans, lead, polycyclic organic matter, and particulate matter from diesel-fueled engines. This paper describes the results of our deliberations on decision-making criteria and reasons for listing these five chemicals. In addition, we describe efforts to evaluate the adequacy of our risk assessment methods to protect infants and children.

741 POTENTIAL IMPACT OF ENVIRONMENTAL EXPOSURES TO POLYCYCLIC ORGANIC MATERIAL (POM) ON CHILDREN'S HEALTH.

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POM, a class of compounds including polycyclic aromatic hydrocarbons (PAHs) and derivatives, e.g. quinones and nitro-PAHs, is listed as a California Toxic Air Contaminant. Sources include various industrial, domestic, and mobile combustion processes. In support of the Children's Environmental Health Protection Act (California SB25), we reviewed POM and PAH toxicity to identify hazards to which infants and children might be especially sensitive. Health standards for POM are typically based on carcinogenicity. Many PAHs are animal carcinogens by various routes. PAH-containing mixtures, such as tobacco smoke, coke-oven fumes, and diesel exhaust, are known human carcinogens. Theoretically, (from both Armitage-Doll and Moolgavkar models) carcinogen exposures early in life lead to greater lifetime cancer risk than later exposures. Experiments have also shown that fetal and neonatal rodents are more sensitive to carcinogenesis by PAHs and PAH derivatives than adults. Studies on pregnant rodents have revealed less widely recognized effects of PAHs, including intrauterine growth retardation (IUGR), fetal mortality, and teratogenesis. Recent studies of populations exposed to industrial air pollution have confirmed the sensitivity of the human fetus to PAH-induced IUGR and dysmorphogenesis. PAHs are toxic to the immune, hematopoietic, and reproductive systems. The toxicity to adult rodents is often reversible and at high doses, but the fetus or neonate may show severe and lasting effects at lower doses. Both cancer and non-cancer toxicity of PAHs generally correlate with the formation of protein and (especially) DNA adducts by reaction with metabolically generated reactive intermediates. Adducts and metabolites have been used as biomarkers of PAH exposure and adverse effect in humans exposed to air pollution and environmental tobacco smoke. Apparently, children may be both more heavily exposed and also more sensitive to the toxic effects of POM.

742 COMPARATIVE STUDY OF THE TOXICITY OF 4-NITROPHENOL AND 2, 4-DINITROPHENOL IN NEWBORN AND YOUNG RATS.

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As infants developing so quickly might be very strongly affected by various environmental circumstances, it is very important to examine the toxicological profiles and levels of chemicals in newborn to lactating animals and compare them with test results using young animals. For the comparative study, we selected 14 phenolic derivatives, of which 4-nitrophenol and 2, 4-dinitrophenol were chosen for evaluation

as the first trial. In the 18-day repeated dose newborn rat study, 4-nitrophenol was orally given from day 4 to day 21 after birth but did not induce any toxicity up to 160 mg/kg, although it induced death in one of six males at 160 mg/kg, and three of six males and one of six females at 230 mg/kg in the dose-finding study. In the 28-day repeated dose oral toxicity study starting at 6 weeks of age, 4-nitrophenol caused death of most males and females at 1, 000 mg/kg but was not toxic at 400 mg/kg except for male rat specific renal toxicity. Unequivocally toxic levels of 4-nitrophenol were considered to be 230 mg/kg/day in newborn rats and 600 to 800 mg/kg/day in young rats, and the NOAELs were 110 mg/kg/day in newborn rats and 400 mg/kg/day in young rats. In the newborn rat study of 2, 4-dinitrophenol, significant lowering of body and organ weights was observed at 20 mg/kg and animals died at 30 mg/kg in the dose-finding study. In the 28-day young rat study, clear toxic signs followed by death occurred at 80 mg/kg but there was no definitive toxicity at 20 mg/kg. Unequivocally toxic levels and NOAELs of 2, 4-dinitrophenol were considered to be 30 and 10 mg/kg/day in newborn rats, and 80 and 20 mg/kg/day in young rats, respectively. Based on these results, it can be concluded that the toxic response of these chemicals in newborn rats is at most 4 times higher than that in young rats.

743 EFFECTS OF PENTYL ETHER, 1, 4-DIETHOXYBUTANE AND 1, 6-DIMETHOXYHEXANE ON MALE RATS FOLLOWING 4-WEEK ORAL ADMINISTRATION.

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Monoethers such as pentyl ether (PE), and diethers such as 1, 4-diethoxybutane (DEB) and 1, 6-dimethoxyhexane (DMH) have been proposed as cetane boosters for diesel fuels. The present study was conducted to assess the short-term oral toxicity of these compounds. Male Sprague-Dawley rats (286 +/- 84 g) were divided into 10 groups of seven animals each. The ethers were dissolved in corn oil and administered by gavage to the animals at dose levels of 2, 20 and 200 mg/kg body weight/day, 5 days per week for 4 weeks. Control animals received corn oil only. No clinical signs of toxicity were observed throughout the treatment period. No significant treatment effects were detected in food consumption and body weight gain. At termination, animals receiving 200 mg/kg of DMH showed marked reduction in the weights of the testis (30%) and thymus (46%) when compared to the control group ($p<0.05$). Significant changes in serum chemistry and hematology were also observed in the 200 mg/kg DMH group only, and consisted of decreased serum uric acid level and lactate dehydrogenase activity, and a 40% reduction in platelet count. No treatment-related changes were detected in N-acetylglucosaminidase activity and protein level in the urine and in the cell-free bronchoalveolar lavage fluids. These results suggest that DMH is more toxic than DEB and PE. Its toxicity in male rats is characterized by testicular and thymic atrophy, and thrombocytopenia.

744 EVALUATION OF INFANT AND CHILD RISK FOR PEROXISOME PROLIFERATORS.

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The evaluation of infant and child risk for the peroxisome proliferator (PP) class of rodent nongenotoxic hepatocarcinogens provides an interesting paradox. Normally, risk is determined from evaluation of the susceptibility of young rodents. However, there are marked species differences in response to PPs and human hepatocytes are resistant to key precursor events such as rodent hepatic peroxisome proliferation and cell proliferation. Here, we present an alternative approach based on human hepatocyte responsiveness to PPs correlated with the donor age. Data on maximal fold change in response to PPs for peroxisome proliferation (measured by cyanide insensitive palmitoyl CoA oxidation) and cell proliferation (measured by 3H thymidine or bromodeoxyuridine incorporation during DNA synthesis) were collated. For peroxisome proliferation, there were 14 donors with an age range from 2 to 54. As expected, the peroxisome proliferation response was weak ranging from 0.1 to 1.4 fold over control. For DNA synthesis, there were 19 donors with an age range from 2 to 26 and, as expected, the induction of DNA synthesis was very weak, ranging from 0.2 to 1.2 fold over control. For both of these key events in PP-induced rodent liver cancer, the correlation of human hepatocyte response with donor age was not statistically significantly different from zero (correlation coefficients of -0.28; p=0.25 and -0.15; p=0.62 for peroxisome proliferation and DNA synthesis, respectively). The power in the calculation gave a high (95%) chance of detecting a correlation of ±0.72 and ±0.80 for cell proliferation and peroxisome proliferation, respectively. These data confirm the resistance to PPs of human hepatocytes isolated from a range of ages of donor, providing evidence for a lack of age-dependent variation in human hepatic response to PPs.

745 MECHANISM BASED APPROACHES TO EVALUATE THE IMPACTS OF LIFE STAGE SPECIFIC EXPOSURES DURING NEURODEVELOPMENT.

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Biologically based Dose-Response (BBDR) models that incorporate mode of action information can improve risk assessment by increasing scientific robustness and applicability across various toxicants and life stages. Towards that goal we are developing and evaluating BBDR models for developmental toxicity based on an underlying hypothesis that rapidly dividing, proliferating and subsequently differentiating cells represent populations potentially sensitive to environmental impacts. Human and rat behavioral, morphological, and cellular studies on ethanol-induced neurodevelopmental toxicity provide a rich data set for application, development, and subsequent assessment of the model. Numerous studies suggest the neocortex is sensitive to ethanol exposure. Previous work modeling ethanol-induced inhibition of rodent neocortical neuronogenesis (G13-G19) has been extended in this current model to include the synaptogenesis period of neurodevelopment (P4-P10). During neocortical synaptogenesis, normal rates of apoptosis range from 30-50% of total neuronal cells affected. With ethanol exposure, dose-dependent increases in these rates are seen (Ikonomidou 2000). At human blood alcohol concentrations that occur after 4-6 drinks (~200 mg/dl), our expanded model predicts an additional 15-20% cellular deficit due to exacerbation of apoptosis during synaptogenesis. Although deficits occurring during the synaptogenesis period are great when the doses are high (> 200 mg/dl), our model suggests the importance of evaluating neuronogenesis at lower, chronic ethanol doses. The extension of our model provides additional mechanistic based approaches to evaluate the impacts of life stage specific exposures during neurodevelopment. Supported by the Center for Child Environmental Health Risks Research through EPA grant R826886 and NIEHS 1PO1ES09601 and the NIEHS Center for Ecogenetics and Environmental Health

746 EVALUATION OF HUMAN DEVELOPMENTAL TOXICITY FOLLOWING PESTICIDE EXPOSURE.

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Federal regulations require that pesticides be tested for reproductive and developmental toxicity in animals prior to registration to establish limits of exposure. Animal data are qualitatively believed to be predictive of human developmental toxicity, but direct comparisons are limited by the availability of adequate human data for specific chemicals. In this study, we identified pesticides with published human reproductive and developmental toxicity data and selected pentachlorophenol (PCP), a chlorophenate pesticide, for evaluation based on the number and type of human studies. A total of 7 epidemiological studies or reports of PCP exposure were identified, including 1 cross-sectional study, 3 case-control studies, 2 cohort studies, and a report of 2 cases. The data were extracted into a database and evaluated for reliability using uniform criteria. Overall, studies were of reliable design, excepting the case reports. Four studies included measurements of PCP in tissues or in environmental samples (indoor air). There was a strong effort in most studies to control for confounders of exposure-outcome relationships. Potential reproductive and developmental outcomes of PCP exposure included reduced birth weight and length, and increased risk of spontaneous abortion, pregnancy complications, altered female hormone levels, congenital anomalies (cataracts), and childhood brain cancer. There appears to be reasonable human evidence that elevated PCP levels are associated with adverse reproductive and developmental outcomes. Existing animal data from standard testing studies will be used to evaluate the predictability for the observed human effects. *Supported in part by an appointment (SAL) to the Postgraduate Research Participation Program at the NCTR administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the USDE and the USFDA. The opinions expressed here are those of the authors and do not necessarily reflect the views or policies of the USEPA or the USFDA.*

747 PHYSIOLOGICALLY BASED PHARMACOKINETIC ANALYSIS OF PERCHLORATE (ClO_4^-) DOSIMETRICS.

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Perchlorate (ClO_4^-) has been found as a contaminant in surface and ground waters of various environmental sites, especially in the western United States. Due to the proven effectiveness of ClO_4^- to cause inhibition of iodide uptake by the thyroid gland of mammals, concern has arisen over the potential health consequences to humans consuming contaminated waters. In order to help quantitate the potential risk to humans, physiologically based pharmacokinetic (PBPK) models have been developed to calculate the absorption, distribution, metabolism and excretion of

ClO_4^- in rats and humans, thereby providing a means of examining the dose-response relationship of ClO_4^- induced iodide inhibition. A PBPK model for ClO_4^- was used to calculate the dosimetrics for ClO_4^- dosed humans, and pregnant and lactating rats, correlated with the inhibition of thyroidal uptake of iodide. Dosimetrics calculated were area under the ClO_4^- time-concentration curve (AUC) in the serum and thyroid, total urinary excreted ClO_4^- in a 24 hr period, peak serum and thyroid ClO_4^- concentrations, and percent inhibition of iodide uptake by the thyroid. A model parameter sensitivity analysis found the AUC of ClO_4^- in serum was most sensitive to the urinary clearance parameter, while the AUC of ClO_4^- in thyroid was most affected by the model parameters controlling uptake of ClO_4^- into the thyroid, plasma binding and transport rates of ClO_4^-. The upregulation (increased symporter Vmax) of ClO_4^- absorption in the thyroid of male rats during chronic ClO_4^- drinking water, hypothesized to be due to increased numbers of NA^+/I^- symporters, had the greatest impact on the interspecies comparison of ClO_4^- dosimetrics.Validated animal and human PBPK models of ClO_4^- kinetics and inhibition of thyroid iodide resulted in the ability to compare ClO_4^- effects in rats *versus* humans, after different exposure routes, both for acute *versus* repeated dosing.

748 STATISTICAL APPROACH TO BRAIN MORPHOMETRY DATA FROM DEVELOPMENTAL NEUROTOXICITY (DNT) TESTING GUIDELINES: PROFILE ANALYSIS.

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Brain morphometry measurements are required in test guidelines proposed by the USEPA to screen chemicals for developmental neurotoxicity. Because the DNT is a screening battery, the analysis of this data should be sensitive to dose-related changes in the pattern of brain growth without requiring prior expectations about specific regions or the magnitude and direction of these changes. In addition, analysis should take into account the lack of independence between multiple brain region measurements taken within the same animal, since the sizes of different regions are likely related. Analysis with t-tests or univariate analyses of variance is inappropriate because it assumes independence and runs the risk of Type 1 error due to repeated sampling using the same error term. A multivariate analysis of variance, profile analysis, was adopted to examine brain morphometry data submitted for the risk assessment of perchlorate (ClO4-), an endocrine-disrupting compound shown to reduce thyroid hormone levels and alter brain development. Profile analysis reduces the number of main effects tested by making between-groups (dose group) comparisons using vectors that comprise all of the within-subject morphometric data from each animal, then comparing these vectors. It also allows gender to be nested within litter, which is necessary to address DNT study designs. The primary test of profile analysis examines whether the pattern made by the various measurements is similar for all treatment groups. This is a test of the interaction between the treatment factor and the within-subjects scores. If the profiles are not parallel, an interaction exists, and further analyses of the interaction factors may be made to elucidate the effects. Results of this analysis showed that ClO4- affected the pattern of brain growth in developing pups at all doses tested. We conclude that this analysis and approach are readily applicable to future DNT data. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

749 QUANTIFYING UNCERTAINTY IN HUMAN REPRODUCTIVE RISK PROJECTED FROM RAT INHALATION STUDIES ON ETHYLENE OXIDE: A FRAMEWORK BASED ON DATA-BASED STATISTICAL DISTRIBUTIONS OF EXTRAPOLATION FACTORS.

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Current noncancer risk assessment does not estimate risk as such but instead computes "safe" doses for the general human population, despite its variation in susceptibility. To account for this variation, and for uncertainty in the extrapolation from animals, "Uncertainty Factors" are applied to animal no-effect levels. Resulting "safe dose" estimates are conservative, but their statistical reliability and the protection actually afforded are poorly characterized. Risks (if any) at exposures above the conservative "safe" levels cannot be addressed, hampering rational risk management analyses. We propose an alternative analytical framework in which the specific sources of extrapolation uncertainty and inter-human variability are characterized as statistical distributions based not on policy choices but on actual data for the observed patterns of variation among cases in instances where they have been characterized. We separately propagate the distributions of extrapolation uncertainties and of human variability to estimate a dose-response curve for humans with empirically supported characterization of the uncertainty in dose levels leading to various levels of human population risk (e.g., 1/100, 1/1000). This allows characterization of the reliability of regulatory levels as "safe" doses and permits estimates of the likelihood of impacts (and lack of impacts) on individuals exposed above such regulatory levels. Using a rich database on the reproductive and developmental toxicity of ethylene oxide in rats, a probabilistic characterization of possible regulatory inhalation thresholds is presented as an example of this dose-response based distributional framework. Our method can be applied to comparative risk analysis and benefit-cost analysis, and it furthers harmonization assessment of carcinogens.

750 GENETIC POLYMORPHISMS IN ASSESSING INTER-INDIVIDUAL VARIABILITY IN DELIVERED DOSE.

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Genetic polymorphisms have been suggested as an important contributor to overall human variability. Recently, these data have been integrated with physiologically-based pharmacokinetic modeling as an approach for determining overall variability. We present an analysis of the potential contribution of polymorphisms in enzymes modulating the disposition of four diverse compounds: warfarin, parathion, methylene chloride, and dichloroacetic acid. Through these case studies, we identify key uncertainties likely to be encountered in the use of polymorphism data, and highlight potential simplifying assumptions that might be required to test the hypothesis that genetic factors are a substantive source of human variability in susceptibility to environmental toxicants. These uncertainties include (1) the relative contribution of multiple enzyme systems, (2) the extent of induction/inhibition through co-exposure, (3) allelic frequencies of major ethnic groups, (4) the absence of chemical-specific phenotype data, (5) large numbers of low-frequency alleles, and (6) uncertainty regarding differences between *in vitro* and *in vivo* kinetic data. Our effort sets the stage for further integration of polymorphism data with PBPK modeling as a means to quantitate population variability

751 RELATIONSHIP BETWEEN TOXICITY VALUES FOR THE HEALTHY SUBPOPULATION AND THE GENERAL POPULATION.

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The application of transport and dispersion models for risk assessment and management requires accurate estimates of human toxicity. For chemical warfare agents (CWA), most of the available toxicological data and estimates were generated in support of their offensive battlefield deployment. The available human toxicity data come from a limited segment of the general population (GP): relatively young, fit, 70 kg, male soldiers. The present military CWA toxicity estimates are not suitable for use with the GP because they are framed for male soldiers and do not consider potentially sensitive members of the GP. A method was created to convert a median effect dosage and probit slope from a military (or healthy subpopulation (HSP)) to a GP. Up until now, the conversion of a military probit slope to a GP probit slope has not been rigorously explored. It was assumed that individual susceptibilities can be modeled as a log-normal distribution. Modifications for gender differences were addressed. Two models were developed to mathematically describe subpopulations (SP) within the GP. The Tail Model assumes that the SP consists of all individuals having susceptibilities within a tail of the GP distribution. The Bell Model assumes that the SP has a log-normal distribution within the larger GP distribution. Historical military demographics were used to evaluated the probable size of the HSP: 20 to 30% of the GP. The ratio of the medians and the ratio of the probit slopes between the SP and GP are easily determined as a function of the SP size. The two models displayed similar trends in ratio values. Uncertainty factors (UF) were calculated based upon the medians of the SP and the GP, and the probit slope of the GP. UF values from this method were consistent with the results of two previous studies that quantified differences between SPs. Qualitative UF estimates used in establishing proposed AEGLs for CWAs require revision in some cases based upon the results of the present study.

752 INCORPORATION OF HUMAN INTERINDIVIDUAL ENZYME EXPRESSION AND BIOTRANSFORMATION VARIANCE INTO HUMAN HEALTH RISK ASSESSMENTS.

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Risk assessments include assumptions about sensitive subpopulations, such as the fraction of the general population that is sensitive and the extent that biochemical or physiological attributes influence sensitivity. Uncertainty factors (UF) account

for both pharmacokinetic (PK) and pharmacodynamic (PD) components, allowing the inclusion of risk-relevant information to replace default assumptions about PK and PD variance (uncertainty). Large numbers of human organ donor samples and recent advances in methods to extrapolate *in vitro* enzyme expression and activity data to the intact human enable the investigation of the impact of PK variance on human susceptibility. The hepatotoxicity of trichloroethylene (TCE) is mediated by acid metabolites whose formation is limited by cytochrome P450 2E1 (CYP2E1) oxidation, and differences in the CYP2E1 expression are hypothesized to affect susceptibility to TCE's liver injury. We combined data sets describing 1) the microsomal protein content of human liver, 2) the CYP2E1 content of human liver microsomal protein, and 3) the *in vitro* Vmax for TCE oxidation by humans. The 5th and 95th percentiles of the resulting distribution differed by 7.6-fold, were converted to mg TCE oxidized/hr/kg body mass and incorporated in a human PBPK model. Simulations of 8 hr inhalation exposure to 50 ppm and oral exposure to 5 g TCE/L in 2L drinking water showed that the amount of TCE oxidized in the liver differs by 2% or less under extreme conditions of CYP2E1 expression and activity. This indicates that differences in CYP2E1 expression among the central 90% of the adult human population account for approximately 2% of the variance of the risk-relevant PK outcome for TCE-mediated liver injury. Integration of *in vitro* metabolism information into physiological models may reduce the uncertainties associated with risk contributions of variance in enzyme expression and the UF that represent PK variance.

753 PBPK MODELING OF BTEX AND N-HEXANE WITHIN A COMPLEX HYDROCARBON MIXTURE: GASOLINE.

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We report an approach for developing PBPK models for gasoline using principal components of toxicological significance. The aim of the study was to determine the pharmacokinetics (PKs) of benzene, toluene, ethyl benzene, and xylene (BTEX) and n-hexane in gasoline, taking enzyme inhibition into account. Due to the mixture's complexity, existing bottom-up modeling approaches were impractical. Instead, we specifically evaluated BTEX and hexane and lumped the remaining components together during both analytical work and PBPK modeling. We conducted closed chamber gas uptake PK studies with F344 rats and monitored the chemical's disappearance from the chamber after treatment with BTEX and n-hexane, or to the whole gasoline, at initial exposure values between 100 and 2500 ppm. Samples of the chamber air were analyzed by gas chromatography/FID using a DB1 capillary column at 35°C and subsequent ramp rate of 25°C/min to 210°C. BTEX and hexane were identified and quantified and the remaining peaks were integrated against a common calibration curve. BTEX and hexane were adequately resolved under these conditions. Each chemical followed a typical gas uptake kinetic decay curve. These data were used to develop a PBPK model for gasoline, including BTEX and hexane as five separate chemicals and all remaining components as a lumped psuedo-chemical. After including previously determined model parameters for BTEX and hexane, kinetic rate estimates for the pseudo chemical were determined through optimization. The model adequately simulated the data for BTEX, hexane, and the lumped gasoline component. This approach appears to be a viable alternative to currently used approaches when complex mixtures are studied. [Supported in part by a Cooperative Agreement from ATSDR (U61/ATU 881475) and the NIEHS Quantitative Toxicology Training Grant T32 ES07321].

754 USING MONTE CARLO SENSITIVITY ANALYSES TO PRIORITIZE DATA NEEDS IN DEVELOPING A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR ACRYLAMIDE.

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Acrylamide is a multi-site carcinogen in rats following chronic, high-dose exposures. Currently, allometric scaling practices used in cancer risk assessment (i.e., interspecies differences in dose according to bodyweight raised to the 3/4 power)do not capture many of the complexities associated with the kinetics of acrylamide. For example, differences between rats and humans have been reported for the formation of hemoglobin adducts following exposure to acrylamide. In an effort to improve risk-based decisions for acrylamide, a PBPK model was developed for acrylamide in the rat using existing data from six published studies. The model provides a good description of the kinetics for acrylamide and its oxidative metabolite, glycidamide. Like many chemicals, uncertainty remains regarding the mode of action by which acrylamide produces its carcinogenic effects. For this reason, Monte Carlo methods were used to perform sensitivity analyses in model predictions for four dose measures, each corresponding to a different assumption for the potential carcinogenic mode of action of acrylamide: (1) genotoxicity; (2) sulfhydryl reactivity; (3) dopamine agonist; and (4) glutathione depletion. The results of the sensitivity analyses indicate that the model parameters for characterizing metabolism *via* cytochrome P450 and glutathione-S-transferase (4-57% of variance), and tissue binding (6-44% of variance) were the most important contributors to variation in dose measures predicted by the model. Although limited human data are available with respect to acrylamide kinetics, the PBPK model presented for acrylamide is useful with respect to (1) organizing existing information on acrylamide kinetics; (2) identifying critical data gaps for risk assessments; and (3) prioritizing the collection of additional data.

755 STATISTICAL ANALYSIS OF PHOTOCOCARCINOGENESIS STUDIES.

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The induction of skin cancer in HRA:SKH-1(hr/hr) hairless mice has been studied for several decades using xenon-arc generated simulated solar light (SSL). This model is currently the most accepted surrogate for sunlight induced skin cancer in humans. Photococarcinogenesis experiments have been used to study the influence of chemicals on SSL induced skin cancer, where a chemical may decrease or increase tumor latency, or alter the number of tumors on the mice. We have compared several statistical methods for analyzing the tumor latency and multiplicity in a study where male and female SKH-1 mice were either untreated or treated with cosmetic vehicle, and exposed to either 600 or 1200 RBU/week of SSL for up to 40 weeks followed by up to 12 weeks of observation. The data were analyzed using a logrank test, negative binomial test, tumor multiplicity test of Dunson, or an adaptation of the single-induction likelihood ratio test of Kodell and Chen. The likelihood ratio, logrank, and negative binomial tests gave similar results with the data. A conclusion from the likelihood ratio test is that the difference between the groups (600 and 1200 RBU/week, no vehicle) was due to a difference in tumor latency (time to observation) and not tumor multiplicity (number of induced tumors/mouse), although the number of tumors per mouse actually observed at any given time was much higher in the 1200 RBU/week group than in the 600 RBU/week group for both sexes. Monte Carlo simulation was used to determine the accuracy of parameter estimates and to evaluate the Type I error rates of the tests. These results demonstrate the utility of these tests in analyzing skin tumor data from similarly designed studies.

756 FROM MICE TO MEN, CANCERS ARE NOT CERTAIN AT OLD AGE.

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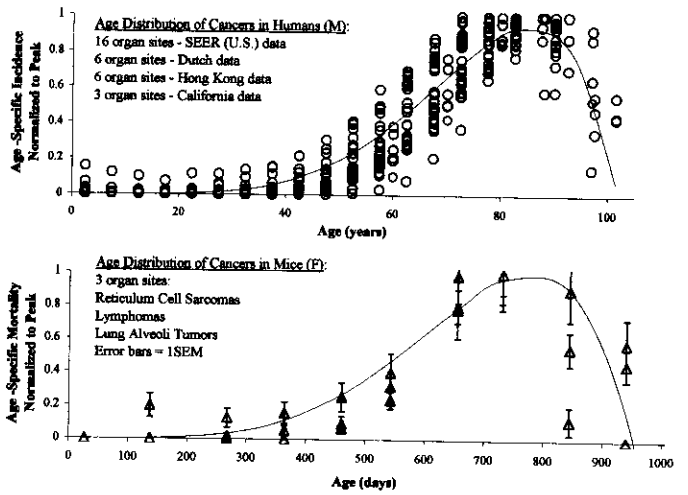
We have studied age-specific incidence data in humans from SEER (US), Holland, Hong Kong, and California and found that organ site incidences do not continue to increase with age, but tend to peak at about age 80, and reduce thereafter toward zero at age about 100. We have also studied cancer incidence (age-specific mortality for lethal cancers) in mice as a function of age in the ED01 undosed controls study cohort ("megamouse" 2-AAF study), where they have been allowed to live very close to their full natural lifetime, and found that similar turnover in incidence occurs at about age 800 days, or 80% of lifetime. A Beta function model [$I(t) = (at)^k e^{(1-bt)}$; a, b, k are constants] fits both the mouse data and the human data well. The model suggests that increasing replicative senescence with age might be the cause of the turnover at old age. Limited data from National Toxicology Program mice studies suggest that the cancer age distribution, including the turnover, may be time-shifted by dietary restriction. Results of a large rat cohort study ("two tons of rats"

nitrosomine study) were inconclusive since the rats did not live long enough for cancer incidence to either turn over, or to reach certainty.



Incidences are normalized to the peak value for each organ site. Organ site incidence data varied over two orders of magnitude for humans, and a factor of three for mice.

757 DNA STRAND BREAKS AND BREAST CANCER RISK.

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Results from human genetic studies suggest that elevated DNA damage and suboptimal DNA repair activity may be associated with an increased breast cancer risk. DNA lesions caused by exogenous and endogenous agents, such as ionizing radiation (IR) and oxygen free radicals, may result in genetic alterations, leading to breast carcinogenesis. Accurate and effective DNA repair mechanisms are essential to maintain genomic integrity. We used the alkaline single-cell gel electrophoresis assay (Comet assay) to evaluate three types of DNA damages (baseline, IR-induced, and damage remaining after a 10-minute repair period) in peripheral lymphocytes of breast cancer cases (n=70) and controls (n=70). The results are presented as the mean comet tail moment of 50 cells using an image analysis system. Breast cancer cases consistently had significantly higher DNA damages compared to controls. Comet tail moments (mean±SD) for cases and controls were: 10.78±3.63 and 6.86±2.76 for baseline, 21.24±4.88 and 14.97±4.18 for IR-induced, and 14.76±5.35 and 9.75±3.35 for damage remaining after repair, respectively. Comet tail moments were independent of established breast cancer risk factors, such as age and family history. Above median baseline, IR-induced DNA damage, and damage remaining after repair were significantly associated with breast cancer risk; age-adjusted odds ratios (ORs) and 95% confidence intervals (CI) were 13.44 (95%CI= 5.97-30.24), 13.65 (95%CI= 6.07-30.71), and 6.54 (95%CI= 3.11-13.79), respectively. Our study suggests that susceptibility to DNA-damaging agents and/or deficient DNA repair may predispose women to breast cancer. A larger study is warranted to further assess the roles of DNA damage/repair in breast cancer susceptibility. Supported by PHS grants CA-73629 and CA-91221.

758 CANCER SLOPE FACTORS FOR IRIS CHEMICALS: COMPARISON OF THE NEW EPA GUIDELINES *VS.* THE TRADITIONAL Q1* METHOD.

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This work compares cancer slope factors for a large number of chemicals calculated by two different methodologies and softwares. In the past, the cancer slope factor has been calculated as the upper 95% confidence limit on the coefficient of the linear term of the multistage model (i.e. q1*) for the extra cancer risk over background. The USEPA's draft cancer guidelines, released in 1999, however, prescribe the cancer slope factor as the slope (s*) of the linear extrapolation between a point of departure (POD) from observed data and the origin of the dose-response curve. This POD is usually the lower 95% confidence limit on dose associated with 10% extra risk adjusted for background. This default in the EPA draft guidelines is thought to provide an upper bound estimate on cancer risk and to incorporate experimental variability. However, the extent of overestimation of risk inherent in this approach has often been raised as an issue. In this work, we examined to what extent the two approaches differed in practice by calculating s* and q1* for 102 carcinogenic chemicals from the IRIS database. In all cases, the constrained multistage model was used to fit the dose-response data using the commercial software Global86 and the EPA Benchmark Dose Software released in 2001. Both softwares obtained identical values of s*. Our results indicate that for 81.4% of the cases, s* is within 0.9 to 1.1 of q1*, and with one exception, the ratio s*:q1* is less than 3.0 for the remaining chemicals. The results validate the argument posed by other authors earlier (Crump and Howe, 1985; Gaylor et al., 1994) that the two approaches are likely to give nearly similar results when the multistage model is used. Our results suggest that for the vast majority of chemicals, the new EPA draft cancer guidelines for the cancer slope factor appears to be neither over nor under protective of public health in comparison with the traditionally estimated q1*. Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the USEPA

759 THEORETICAL MODELS FOR DNA ADDUCT FORMATION AND *HPRT* MUTATION INDUCTION BY LOW-DOSE ETHYLENE OXIDE EXPOSURE OF MICE AND RATS.

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In order to evaluate the effects of low-dose ethylene oxide (EO) exposures upon the induction of N7-(2-hydroxyethyl)guanine (7-HEG) and *Hprt* mutations, we characterized previously published data using theoretical models. These adduct and mutant frequency (MF) data were from male B6C3F1 mice and F344 rats exposed by inhalation for 4 weeks (6 h/day, 5 days/week) to 0, 3, 10, or 33 ppm EO (Wu et al., Carcinogenesis 20:1787-1792, 1999) or to 40, 1000, or 3000 ppm ethylene (Walker et al., Carcinogenesis 21:1661-1669, 2000). The Dose-Response curves for 7-HEG formation in liver, lung, brain, and spleen were adequately characterized using a power function (PF) model expressed as $y = \alpha C^{\beta}$, where C represents the concentration of EO and α and β are positive parameters which reflect the relative sensitivity of the tissue to EO-induced DNA damage. A value of $\beta = 1$ corresponds to the linear-no-threshold (LNT) model. Application of the PF model showed that, for the 7-HEG Dose-Response curves for EO-exposed mice, $\beta > 1$; while, for the corresponding curves for EO-exposed rats, $\beta < 1$. Thus, use of the LNT model to estimate adduct formation will overestimate the risk for EO-induced DNA damage in mouse tissues, but will underestimate the risk for DNA damage in rat tissues. The Dose-Response curve for EO-induced *Hprt* MFs in T-lymphocytes from exposed mice has been previously shown to be sub-linear (i.e., to have a lesser effect per unit dose of EO as the exposure concentration was lowered) (Walker et al., Mutat. Res. 392:211-222, 1997). We applied a mechanistic based model called NEOTRANS2-EO to induced MFs in ethylene/EO-exposed mice, and this model predicted that the threshold for the induction of excess mutations (i.e., over background) to be approximately 10 ppm EO. These analyses indicate that, at low doses of EO, the Dose-Response curves for 7-HEG and *Hprt* MFs in rat and mouse do not follow the LNT model. (Supported in part by the Department of Energy Office of Science and Environmental Management).

760 VINYL ACETATE-INDUCED INTRACELLULAR ACIDIFICATION: PROOF OF CONCEPT FOR MODE OF ACTION.

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Inhaled vinyl acetate (VA) causes epithelial degeneration, cell proliferation and tumors in the nasal cavity of rats, but not mice. Carboxylesterase (CE) catalyzes hydrolysis of VA to acetaldehyde and acetic acid. Acetaldehyde is further oxidized to acetic acid with a stoichiometric yield of 3 protons. Intracellular acidification is hypothesized to be the critical first step preceding cytotoxicity. The purpose of these experiments was to demonstrate that exposure of CE-containing cells to VA causes reduction in intracellular pH (pHi). Primary rat hepatocytes were chosen as a model cell system because they contain CE activity comparable to rat nasal epithelium. Freshly isolated rat hepatocytes were attached to polylysine-coated glass coverslips and loaded with the intracellular dye BCECF-AM. Quantitative fluorescence imaging microscopy was used to monitor pHi in response to various concentrations of vinyl acetate. Concentrations of 10 µM - 800 µM VA produced rapid (≈ 70 sec.) decrements in pHi (Δ=-0.03 —0.53) that were rapidly reversible (≈ 70 sec.) upon removal of VA. Pretreatment of hepatocytes with bis(p-nitrophenyl)phosphate, a specific CE inhibitor attenuated the VA-induced intracellular acidification. These results support a mechanism of VA carcinogenesis involving intracellular acidification, cytotoxicity and cell proliferation and, together with other quantitative toxicity and dosimetry information, suggest a margin of exposure-based approach to risk assessment. Supported in part by the Vinyl Acetate Toxicology Group, Inc..

761 STRAIN-SPECIFIC DIFFERENCES IN SUPEROXIDE DISMUTASE AND 8-OXOGUANOSINE DNA-GLYCOSYLASE CAPACITY OF MALE C57BL/J, BALB/C, AND ICR MICE.

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Regulatory agencies use a variety of methods to estimate the risks to humans from exposures to toxic agents. Many of these methods are based on toxicological data derived from animal models, particularly rodents, and have limitations in their ability to predict human risk. The purpose of this study was to characterize the differences that exist in the enzymatic capacity of key enzymes responsible for the clearance of superoxide anion and the oxidized DNA base 8-hydroxy-2'-deoxyguanosine (oxo8dG), superoxide dismutase (SOD) and 8-oxoguanosine DNA-glycosylase (Ogg1), respectively. Brain regions, cerebellum and caudate putamen, from male C57BL/J, Balb/c, and ICR mice were analyzed. There was no significant difference between strains in the activity or protein level of Ogg1 in the cerebellum; however, the activity of Ogg1 in the caudate putamen of ICR mice was 34% and 31% lower than in C57BL/J and Balb/c, respectively. SOD activity in the cerebellum was twice as high in C57BL/J mice *versus* Balb/c or ICR. SOD activity in the caudate putamen of C57BL/J mice was four fold higher than in Balb/c and ICR. The functional relevance of these differences was determined by measuring levels of oxo8dG, an oxidized 2-deoxyguanosine formed by reactive oxygen species, such as superoxide anion. The levels of oxo8dG in the cerebellum and caudate putamen of C57BL/J mice were nearly twice as high as the respective regions in Balb/c and ICR mice. Based on these results, it is argued that risk estimations based on animal bioassay data may be unnecessarily high in some cases and, in others, there may be greater uncertainty. (Supported in part by a VAMR Grant, the Japan Society for the Promotion of Science, and a US Department. of Defense Grant)

762 TRANSGENIC DATA AS PREDICTORS OF CARCINOGENICITY.

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Sponsor: C. Abernathy.

Studies of the tumorigenic potential of chemicals conducted in transgenic species offer several advantages. The transgenic strains carry genetic weaknesses that increase and shorten the time to tumor response thereby lessening cost and time for carcinogenicity assays. At the same time, observations of responses in transgenic animals increase our knowledge of the integrated impact of their genetic defects on the cancer mode of action. However, the limited data from transgenic studies makes it difficult to determine how transgenic results should be applied in weight of evidence determinations for carcinogenicity. EPA examined the concordance between the results from studies of several transgenic strains (p53+/-, Tg.AC, Tg rasH2, E -pim-1, and XPA-/- transgenic mouse strains) and the results from standard bioassays. The findings demonstrate the promise that transgenic data may offer an efficient option for identifying potential carcinogens without the necessity of conducting a bioassay in rodent species. However, it will be important to continue to build a database of transgenic data in order to refine its usefulness as a predictive tool. [The opinions expressed in this abstract are those of the authors and not necessarily those of USEPA.]

763 AREA-UNDER-THE-CURVE (AUC) AS A DOSE METRIC FOR PROMOTIONAL RESPONSES FOLLOWING 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) EXPOSURE.

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An underlying basis of risk assessment is that an equivalent risk for a certain dose metric exists that allows for extrapolation between species. To better understand the use of AUC as a dose metric for promotional responses following TCDD exposure, a novel study was designed using a newly revised physiologically-based pharmacokinetic model in the female Sprague-Dawley rat to obtain similar liver AUCs of TCDD by different patterns of exposure. In the first TCDD-treated group, rats received an administered average daily dose of 100 ng/kg/day TCDD in corn oil for the study duration of 15 weeks. In the second TCDD group, rats received a high peak dose averaging 1000 ng/kg/day for the first week followed by a lower TCDD administered dose of 23 ng/kg/day for the remainder of the study. Age-matched control rats received corn oil as a vehicle control. Altered hepatic foci (AHF) positive for the placental form of glutatione-*S*-transferase (PGST) enzyme were measured from representative lobes of the liver stained immunohistochemically for PGST expression. Volume fraction, expressed as a percentage, is considered to be the most stable measure of AHF. The median volume fraction was 0.045% in control rats and was significantly elevated in both TCDD treatment groups. However, the volume fraction of PGST-positive foci was significantly higher in the TCDD group given the high peak dose during the first week of TCDD treatment compared with the group receiving the same average daily dose over the study duration: 0.74% *vs.* 0.17%, respectively. This study also confirmed previous analyses that tissue burden of TCDD is not an appropriate dose metric for the promotional responses induced by TCDD following exposure. These findings suggest that the peak magnitude of TCDD in liver may play a significant role in additional to AUC in the induction of promotional responses by TCDD.

764 DOSE DEPENDENT LIVER REGENERATION FOLLOWING CO-ADMINISTRATION OF CHLOROFORM AND TRICHLOROETHYLENE-INDUCED HEPATOTOXICITY IN MALE SPRAGUE-DAWLEY RATS.

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Chloroform ($CHCl_3$) and trichloroethylene (TCE) are common contaminants present in drinking water and regarded as hepatotoxicants. The aim of this study was to develop a dose-response relationship for $CHCl_3$ + TCE binary mixture (BM) by measuring liver injury and tissue repair and to compare the previous results with CHCl3 and TCE alone. Male Sprague-Dawley rats (250-300g) were administered with 5-fold dose range of both $CHCl_3$ (74 to 370mg/kg, ip) and TCE (250 to 1250mg/kg, ip) in corn oil (maximum of 0.5ml/kg). Liver injury was assessed by plasma ALT and AST activities and tissue repair was measured by ^{3}H-thymidine incorporation into hepatonuclear DNA. Since both these compounds are known to cause nephrotoxicity, blood urea nitrogen (BUN) levels were assayed to evaluate kidney damage. Levels of both the parent compounds and two major metabolites of TCE [trichloroacetic acid (TCA) and trichloroethanol (TCOH)] were quantified by gas chromatography. The levels of $CHCl_3$ peaked in the liver and blood at 60 min and declined thereafter. $CHCl_3$levels were higher after injection of BM compared to injection of $CHCl_3$ alone. Liver injury, as assessed by ALT and AST, was higher at early time points and subsequently regressed. However, dose-response was not evident, possibly due to the inhibition of these plasma transaminases by TCA and TCOH, metabolites of TCE. Tissue repair response followed a dose-dependent increase at all time points, and was evident at 24 h and peaked at 48 h. At 24 h, only the highest combination caused kidney damage. The $CHCl_3$ + TCE BM caused either subadditive or no more than additive hepatotoxicity. Thus, the present results support the concept that tissue repair response is critical in restraining the progression of injury (supported by ATSDR U61/ATD 681482).

765 CHRONIC RISK ASSESSMENT FOR TRIBUTYL PHOSPHATE (CAS NO. 126-73-8).

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Tributyl phosphate (TBP) is a non-flammable, non-explosive liquid that is used as a solvent or an anti-foaming agent. Dietary exposure to tributyl phosphate at high doses produces a reversible hyperplasia of the urinary bladder epithelium which is not due to the presence of crystalluria, urinary precipitates or calculi, but may possibly be attributed to the presence of a toxic metabolite(s), tertiary butyl alcohol and or dibutyl hydrogen phosphate. A 2-year study of rats and mice identified both a NOAEL and LOAEL for bladder hyperplasia in rats, and was selected as the key study for the derivation of the provisional RfD. Mice did not show significant dose-response in any of the target organs examined in this 2-year chronic study. The 2-year rat study reported a significant increase of urinary bladder papillomas and carcinomas in males and urinary bladder papillomas in females, but only in animals exposed to the highest level of TBP in their diet. There is no clear evidence for the mode(s) of action for tributyl phosphate-induced urinary bladder tumors in rats. The vast majority of *in vitro* and *in vivo* tests indicate that tributyl phosphate is negative for genotoxicity. The apparent lack of genotoxic effects, coupled with low-dose hyperplasia and high-dose tumor formation, is suggestive of a non-genotoxic mechanism of carcinogenesis. Therefore, several different approaches were used when calculating a quantitative estimate of carcinogenic risk. Provisional oral slope factors were derived using both a linearized multistage model and a margin of exposure analysis, and the results produced by the different methods were compared. (This abstract does not necessarily reflect EPA policy).

766 PROPOSED ORAL SLOPE FACTOR FOR P-CHLOROANILINE UTILIZING LINEAR AND NON-LINEAR APPROACHES.

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A review of the available carcinogenicity data for p-chloroaniline identified the chemical as a candidate for analysis of carcinogenic risk by both the traditional linear method as well as the proposed nonlinear (margin of exposure) method. Although no human carcinogenicity data are available, published chronic oral studies in rats and mice provide evidence of multi-organ carcinogenicity. The cancer risk assessment for p-chloroaniline is complicated by species differences in target organ susceptibility and uncertainty as to which of the compound's possible carcinogenic mechanisms is most relevant to humans. There is evidence for genotoxic and non-genotoxic modes of action, both of which apparently require bioactivation of p-chloroaniline. The primary tumor types observed in the oral studies are rare splenic tumors (fibrosarcoma, osteosarcoma, or hemangiosarcoma) and adrenal medullary pheochromocytomas in male rats, and hepatocellular carcinomas in male mice. Data on tissue-specific distributions of reductase activities, which protect against the reactive intermediates of p-chloroaniline, suggest that the mouse hepatocellular tumors may not be relevant to humans. However, either of the rat tumors could be relevant to humans: the adrenal tumors induced by a genotoxic mechanism, and the splenic tumors induced by a non-genotoxic mechanism secondary to erythrocyte toxicity. Dose-response modeling of the incidence data for rat tumors was performed using both linear and non-linear methods. In deriving oral slope factors based on rat adrenal and splenic tumors, the default approach assuming linearity was compared with the margin of exposure approach, which assumes nonlinearity. The margin of exposure approach for splenic sarcomas was based on the assumption that an oral dose of p-chloroaniline that was protective for splenic fibrosis (a non-neoplastic lesion) would also be protective for carcinogenicity in the spleen.

767 ORAL SLOPE FACTOR FOR BUTYL BENZYL PHTHALATE (BBP): COMPARISON OF A LINEAR AND A NONLINEAR APPROACH.

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A review of the available carcinogenicity data for BBP (CASRN 85-68-7) identified the chemical as a candidate for analysis of carcinogenic risk by both the traditional linear method as well as the proposed nonlinear (margin of exposure) method. Currently, BBP is classified as Group C (Possible Human Carcinogen) based on no human data and limited animal data. The basis for this classification is a 1982 NTP study wherein a statistically significant increase in mononuclear cell leukemia was reported in female rats following two years of exposure to 12, 000 ppm of BBP in the feed. A quantitative estimate of carcinogenic risk from oral exposure is not currently included on IRIS due to the qualitative weakness of the response, including similar pathologies in the control and treated groups and lack of reduction in time to first tumor. More recently, NTP conducted additional long-term feeding studies with BBP in F344 rats. The results of these studies provided some evidence of an increase in the incidence of pancreatic cancer in male F344 rats, though a significant increase was only seen at the highest exposure level (12, 000 ppm). Concurrent with these neoplastic lesions, the study reported a dose-dependent increase in preneoplastic lesions (acinar hyperplasia) in the pancreas. The mode(s) of action of BBP-induced carcinogenesis are not clear. There is limited evidence supporting both genotoxic and non-genotoxic mechanisms. The majority of data from genotoxicity testing have indicated that BBP is not strongly genotoxic. Moreover, BBP, like other phthalate diesters, induces peroxisome proliferation, which is the putative mechanism of action for the observed changes in the pancreas. A comparison of the results using the default linear approach and a nonlinear (margin of exposure) approach provides insight into the effect of linearity assumptions in deriving of quantitative estimates of oral carcinogenic risk for BBP.

768 QUANTITATIVE RISK ASSESSMENT OF INHALED REFRACTORY CERAMIC FIBERS (RCF).

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Refractory ceramic fibers(RCF) are man-made vitreous fibers primarily used in industrial settings as a lining in high-temperature furnaces, heaters, and kilns. Occupational studies have noted, among workers in the RCF industry, decreases in lung function and an increased rate of pleural plaques. A multi-dose chronic inhalation study of RCF in rats has shown a dose-related response with respect to the development of chronic inflammation, fibrosis and cancer of the respiratory system. Using mathematical models that were developed for dosimetric extrapolation of the observed animal exposure-dose-response data to humans, quantitative risk assessments for cancer and non-cancer effects of the respiratory system in humans were conducted. For cancer risk assessment, a linearized multi-stage model was used which appears to be compatible with the rat lung tumor response data. The dose metric used was the number of retained WHO (World Health Organization) fibers (>5μm in length)in the lung. For non-cancer effects, a benchmark dose approach was used for deriving a reference concentration (RfC) for environmental RCF exposure. The dose metric used was the surface area of retained total fibers in the lung. Additional uncertainty factors of 3 and 10 for rat to human extrapolation and for more sensitive members of the population, respectively, were applied for estimating the RfC. The 95% upper-bound estimates of human cancer risk were 4.76 x 10^{-3} per airborne WHO fiber/cm^3 for continuous environmental exposure and 2.36 x 10^{-3} for intermittent occupational exposures. The RfC for interstitial fibrosis was 0.2 total fibers/cm^3 for continuous environmental RCF exposure. (The views and opinions expressed by the authors do not necessarily reflect the official position or policy of the USEPA.)

769 CARBON BLACK SHOULD NOT BE CLASSIFIED AS A HUMAN CARCINOGEN.

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In the 1996 monograph on carbon black (CB), IARC classified CB as a group 2B carcinogen, possibly carcinogenic to humans. In 1990, the State of California EPA listed CB extracts as a chemical known to the State to cause cancer. There is intent to list CB as well. We have reviewed the 1996 IARC monograph as well as data published subsequent to its release and conclude that CB should not be classified as a human carcinogen. CB has been shown to be carcinogenic to rats but not to mice, hamsters, guinea pigs or primates even when lung burdens are several times higher then the lowest tumor-inducing particle lung loads in rats. Studies conducted by inhalation and intratracheal administration to rats have shown significant increases in benign and malignant lung tumors and lesions described as benign cystic keratinizing squamous-cell (KSC) tumors. Most mutagenicity studies have been negative and therefore it appears that CB is not carcinogenic to rats *via* a genetic mechanism. Studies carried out to demonstrate a possible epigenetic mechanism have shown that in the presence of significant inflammation a significant increase in DNA adducts in alveolar type II cells and hprt (hypoxanthine-phosphoribosyltransferase) mutations occurs. It is postulated that the severe inflammation causes oxidant release which in turn causes DNA damage with resulting tumor formation. Because of the unique response by the rat to CB exposure, the rat bioassay should not be used to conduct a risk assessment. Lung tumor formation, including KSC lesions, occur only in rats. An expert panel reviewing KSC lesions induced by TiO_2 or p-aramid agreed that similar lesions are not seen in humans either. The location of lung tumors in rats and humans differs as well. When lung tumors occur in humans they are primarily located in the bronchial airways, whereas in the rat they occur in the parenchyma and are alveolar in origin. Epidemiology studies have failed to adequately demonstrate an increase risk of lung cancer due to occupational exposure to CB. Therefore, CB should not be classified as carcinogenic to humans.

770 ASSESSMENT OF "ALL CANCERS" IN DIOXIN EPIDEMIOLOGY STUDIES.

L. A. Beyer, B. D. Beck and K. W. Chan. *Gradient Corporation, Cambridge, MA.*

The carcinogenicity of dioxin in humans is a source of vigorous scientific debate. As a result, different organizations classify its carcinogenicity differently. For example, USEPA recently recommended that dioxin be classified as a human carcinogen. In contrast, the majority of members on USEPA's Science Advisory Board's Dioxin Reassessment Review Subcommittee did not support the classification of TCDD as a human carcinogen. It is therefore useful to compare results of the various epidemiology studies, which evaluated the cancer incidence/mortality in populations exposed to relatively large concentrations of dioxin. We selected the epidemiology studies, based on size of population, length of followup, and characterization of exposure, and summarized their findings for the category "all cancers." These studies involved nine occupational cohorts from a number of countries including the US, the Netherlands, and Germany; and one residential cohort in Italy (Seveso). We restricted our analysis to "all cancers, " the cancer increase relied on by USEPA as the basis for designating dioxin as a carcinogen. The results show that the relative risks are small or nonexistent even after accidents associated with high exposure levels. For example, in Bertazzi et al. (2001), a study of residents exposed to dioxin in Seveso, Italy, "all cancers" are not increased in the zone A (highest exposed) population in either men and women combined, or in women or men separately (overall or for any time interval since first exposure). In occupational studies, the relative risks are relatively small and mixed: Becher et al. (1996), in a study of four occupational cohorts, found a significant increase in only one of the four cohorts. The increase was relatively small (standardized mortality ratio (SMR) = 134), and was

found in the cohort with the shortest mean duration of employment. Therefore we conclude that, based on epidemiology studies, the evidence for an association between dioxin and excess total cancer in humans is mixed, at best.

771 ASSESSMENT OF BENZENE CARCINOGENIC POTENTIAL IN HUMANS.

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Benzene is currently classified as a human carcinogen based primarily on increased risks of leukemia, with acute myeloid leukemia as the predominant form. Recently, some researchers have suggested that benzene also causes other types of cancer such as, lymphoma, non-Hodgkin's lymphoma, multiple myeloma, brain, lung, and kidney cancers. It is therefore of interest to assess the evidence for the carcinogenicity of benzene with respect to these other tumor sites. We selected seven epidemiological studies determined to be of sufficient quality, based on analysis of specific cancer outcomes, assessment of benzene exposure, and cohort size of more than 300 workers. These studies involved cohort studies of workers in painting, printing, painting and shoe-manufacturing, chemical production, oil refinery, and workers as car and mobile equipment mechanics, and filling station managers in China, Italy, Sweden, United Kingdom, and United States. While excess lymphoma, non-Hodgkin's lymphoma, and lung cancer were observed in a group of benzene-exposed Chinese workers, five other studies did not demonstrate any significant increase in risks among other workers exposed to benzene or benzene-containing petroleum products. Findings in multiple myeloma, brain and kidney cancers in workers were also inconclusive, for example, there was significant increased risk of brain cancer in small service filling stations, but not in large service filling stations, and two other studies did not demonstrate any significant increased risk of brain cancer in workers exposed to benzene. Therefore, we conclude that there is no convincing evidence for an association between benzene and the tumor types noted above.

772 AN ASSESSMENT OF THE HUMAN RISKS ASSOCIATED WITH RADIOFREQUENCY EXPOSURE.

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Many emerging technologies depend on radiofrequency (RF) electromagnetic (EM) energy; consequently, public concern regarding potential hazards associated with RF exposure is high. To assess the human health effects of low-level RF exposure, 80 epidemiology studies published since 1965 related to EM radiation exposure were identified; these were categorized according to EM frequency ranges (0-300 kHz, 0.3-30 MHz, and 0.03-300 GHz). The majority of studies identified dealt with cancer endpoints. Within each EM frequency category, studies were further classified according to study design (cohort, ecological, case-control, etc). Studies of exposures in the 0.03-300 GHz range (the RF range) were given greatest weight in assessing potential health risks; these included eight cohort studies, four ecological analyses, twelve case-control studies, and one cross-sectional report. Overall, the individual epidemiology studies related to EM frequency exposures are deficient for risk assessment purposes; they suffer from methodological flaws, design biases, and inadequate exposure measurements. Analyzed as a whole using the Hill criteria for causation, these studies do not provide evidence of adverse health outcomes related to low-level RF exposure. Those studies that reported potentially increased health risks associated with RF exposure (primarily brain and hematopoietic cancers) failed to demonstrate consistent exposure-response relationships and showed disparate findings that were not confirmed in other studies. The studies related to the 0-300 kHz and 0.3-30 MHz frequency ranges also reported discordant findings with no consistent exposure-response data. The lack of epidemiological evidence for potentially adverse human health outcomes related to RF exposure is supported by animal data and mechanistic studies which demonstrate that temperature increases in exposed tissues (which do not occur at exposure levels below current US regulatory limits) are required to cause biological effects. In summary, the available epidemiology data suggest that exposure to low-level RF is not associated with adverse human health effects.

773 LUNG CANCER MORTALITY AMONG WORKERS EXPOSED TO AIRBORNE HEXAVALENT CHROMIUM.

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This study assesses mortality through 1997 for 492 persons who worked at a chromate production plant for at least one year between 1940 and 1972. Cumulative airborne Cr(VI) exposures were quantified for each worker and, together with this mortality assessment, provide new data for use in cancer risk assessment. Cohort members were followed for mortality through 1997, and standardized mortality ratios (SMRs) were estimated. Lung cancer mortality was investigated further by calculating SMRs stratified by duration of employment, time since hire, year of hire, and categories of cumulative exposure to Cr(VI). Including 51 deaths due to lung cancer, 303 deaths occurred. SMRs were significantly elevated for all causes (SMR=129, 95% CI 115–144), all cancers (SMR=155, 95% CI 125–191), and lung cancer (SMR=241, 95% CI 180–317). Lung cancer mortality was significantly elevated for the highest two cumulative exposure groups—>1.05 to <2.70 mg/m^3-years, SMR=365, 95% CI 208—592; and >2.71 to <29.0 mg/m^3-years, SMR=463, 95% CI 283–716—but not for the lowest three exposure groups. Significantly elevated SMRs were also found for those hired before 1960, with 20 or more years of employment, and with latency of 20 or more years. No excess lung cancer was found among workers first employed after 1960, which may reflect the conversion to a lower-lime process and lower exposures due to improved industrial hygiene. The stratified analysis of lung cancer mortality by cumulative exposure suggests a threshold effect, because risk is statistically significantly elevated only at exposure levels over 1.05 mg/m^3-years. Although a threshold is consistent with recently published toxicological studies, this finding must be interpreted cautiously, because the data are also consistent with a linear Dose-Response, perhaps due to the small numbers of lung cancer deaths in the lowest exposure groups.

774 DOSE-RESPONSE ASSESSMENT FOR LUNG CANCER MORTALITY OF AN OCCUPATIONAL COHORT EXPOSED TO AIRBORNE HEXAVALENT CHROMIUM.

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The dose-response relationship for inhalation exposure to hexavalent chromium [Cr(VI)] and lung cancer mortality is assessed for persons who worked at a chromate production facility from 1940 through 1972. Dose metrics of maximum 8-hour time-weighted-average (TWA) exposure, defined as the highest TWA exposure estimated for each worker, and lifetime cumulative exposure (e.g., mg/m^3-years) were evaluated. Poisson and Cox regression models were used to estimate a benchmark dose for 10% additional lifetime risk and unit risks (risk from exposure to 1 µg/m^3) for occupational and continuous (e.g., environmental) exposure scenarios. Trend analyses were performed to identify the highest exposure level for which there is statistical evidence of an increased risk of lung cancer. Cumulative exposure to Cr(VI) ranged from 0 to 29 mg/m^3-years, and the maximum TWA exposures ranged from 3 µg/m^3 to 5, 518 µg/m^3. A linear dose-response was observed in both of these exposure ranges. The benchmark dose for continuous exposures was 10 µg/m^3, and the maximum likelihood estimate of unit risk was 0.011 µg/m^3. However, the dose-response was also consistent with a threshold for both dose metrics. There was no statistical evidence of an increased cancer risk at maximum occupational TWA exposures of 116 µg/m^3, or cumulative exposures of 0.8 mg/m^3-years, corresponding to a 40-year occupational exposure level of 20 µg/m^3. The estimated unit risks are approximately equal to those developed by the USEPA in 1984, and are two to six times lower than those proposed by OSHA in 1995. The absence of a linear trend below certain doses suggests a threshold effect although a non-threshold dose-response cannot be ruled out. The lack of an increased cancer risk at low exposure levels is consistent with mechanistic data suggesting that at low exposure levels, Cr(VI) is reduced to and detoxified by extra- and inter-cellular components of the lung.

775 USING QSAR IN THE SELECTION OF SURROGATE TOXICITY VALUE.

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The National Center for Environmental Assessment, Cincinnati (NCEA-Cin), through it's Superfund Technical Support Center (STSC), provides rapid-turnaround technical support to Regional staff and others for time-critical decision making under the Superfund program. A key element of this support is the development of provisional toxicity values, such as oral Reference Doses (RfDs), inhalation Reference Concentrations (RfCs), oral cancer slope factors (SFOs), or inhalation cancer unit risks (URIs). When the published data are inadequate for the development of toxicity values, the Center makes an attempt to identify an appropriate surrogate using a quantitative structure activity relationship (QSAR) approach. A commercial QSAR model, TOPKAT®, is utilized to establish a degree of structural similarity between the query chemical (chemical under examination) and the compounds in the models database. The two models utilized in the selection process are the Oral Rat Chronic LOAEL and the Rat Oral LD_{50} models. The

query chemical is initially queried using the LOAEL model. Once a valid prediction is obtained (i. e., if all model requirements are satisfactorily met) a "similarity search" is conducted which determines the database compounds most similar to the query compound in terms of common descriptors. All database chemicals that satisfy the similarity distance of 0.200 (distance judged by TOPKAT® modelers to represent most similar compounds) are considered to be potential surrogates and are then evaluated for toxicity values on IRIS, HEAST or Provisional Toxicity Value databases. If the query chemical does not satisfy all model considerations for the LOAEL model a valid prediction is not obtained and the chemical is queried utilizing the LD_{50} model to determine a possible surrogate. An example of this selection process will be presented using dichlorobenzophenone (DCBP) a metabolite of chlorobenzilate (CB). This abstract does not necessarily reflect EPA policy or endorsement

776 A COMPARISON OF SIX DOSE-RESPONSE MODELS FOR USE IN QUANTITATIVE RISK ASSESSMENT OF WATERBORNE PATHOGENS.

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Assessment of risk from waterborne pathogens requires the development of a dose-response model to characterize the relationship between the level of microbial exposure and the probability of a particular adverse consequence (i.e., infection/illness). A properly chosen dose-response model enables us to estimate the individual infectious dose (ID) necessary to cause infection or illness in a percentage of the population. Several models have been used in the literature to describe microbial dose-response data. These include exponential and beta-Poisson models, which are considered biologically plausible models; log-logistic, log-probit and Weibull models, which are considered empirical models; and the Weibull-gamma model. There is no consensus on which model is most applicable to microbial dose-response assessment, although the biologically plausible models are favored by many authors. In selecting a dose-response model for microbial risk assessment, we recommend considering the following: (1) goodness-of-fit, (2) simplicity in model formulation, without sacrificing goodness of fit, and (3) conservative prediction of infectious dose at low levels. Each dose-response model was fitted to data collected by Schiff et al (1984) for echovirus 12 administered to healthy human volunteers in nonchlorinated water. The beta-Poisson, log-logistic, log-probit, and Weibull models all provided good fits to the data, with the estimated 1% IDs ranging from 1.7 to 17.0 PFU for these models. The most conservative estimate of 1.7 PFU from the Weibull model was at least four-fold less than doses predicted by the other models, although a bootstrap 95% confidence interval for the 1% ID from this model (0.06, 18.6) included all of the estimated values from the other models. Although not a formal test, this suggests that the model predictions were not substantially different.

777 DEVELOPMENT OF A TOXICOLOGY DATABASE FOR THE PREDICTION OF TOXICITY: AN ILSI/HESI STRUCTURE ACTIVITY RELATIONSHIP PROJECT.

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Scientists need to rapidly predict the toxicity of new compounds using computerized models based on quantitative structure activity relationship (QSAR) or other techniques. The predictive ability of these models will largely depend on the availability of reliable data for sufficient compounds covering a broad array of chemical categories. An international collaboration of the ILSI Health and Environmental Sciences Institute and LHASA, at the University of Leeds (UK), has developed a pilot database of toxicity testing data. The collaboration also includes global industrial manufacturing organizations in the consumer products, chemicals, and pharmaceutical sectors, as well as representatives from international regulatory agencies. The pilot database has been populated with toxicology data for four endpoints - carcinogenicity, mutagenicity, dermal sensitization and hepatic toxicity compiled from publicly available sources. The pilot database was derived from the European Chemical Bureau's (ECB) International Uniform Chemical Database (IUCLID) system, and the database can be used for structure or substructure searches and biological data. The database will be further populated with data from additional toxicological and environmental endpoints. The database will be made publicly available for development and improvement of predictive toxicological models, and as a screening tool for early chemical hazard identification. The benefits of this project include increased reliability and speed of prediction of toxicity for new chemical moieties, e.g., pharmaceuticals, cosmetic ingredients, etc., and a potential reduction in animal testing.

778 STATISTICAL METHODS FOR ANALYZING COMMUNITY INTERVENTION STUDIES: APPROACHES FOR EVALUATING SAMPLES BELOW DETECTION LIMITS.

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Interpretation of data below detection limits is always problematic because most methods of statistical analysis assume that all values are observed. When the fraction of samples below a detection limit is 10% or less, the common recommendations to substitute half of the detection limit or 0 for non-detects which allows reasonable use of many statistical methods. When a large fraction of the measurements are below a detection limit a more robust procedure is needed. A number of statistical methods have been developed to analyze this type of data, but often are not easily applied by investigators. We have developed simplified methods for simulating the data below detection limits such that standard methods of statistical analysis can be applied. These methods assume that the shape of the distribution of values above the detection limit provides information about the shape of the distribution below a detection limit through the use of quantile-quantile plots. We applied these methods to analyze data on organophosphorus pesticide metabolites in urine from children in a community intervention study. The pesticide metabolite data had multiple detection limits and groups of metabolites were summed for estimates of exposures. The results illustrate that in complex situations the proposed method has many advantages in reducing bias compared to the usual recommendations for dealing with measurements below a detection level, and provides better estimates of confidence intervals. Validation of the new methods through simulation studies showed that the reduction of bias by the proposed method compared to usual methods can be over 90% when non-detects are 95% of the samples, and that adjusted 95% confidence levels contain the true value 90% to 96% of the time. (Supported by Center for Child Environmental Health Risk Research through grants EPA R82688601, and NIEHS 5PO1ES09601-05, and by National Research Center for Statistics and the Environment through grant EPA CR825173.)

779 HIERARCHICAL CLUSTER ANALYSIS OF NEUROLOGICALLY RELATED ADVERSE EVENTS ASSOCIATED WITH THERAPEUTIC USE OF DRUGS.

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We extracted neurologically related data from the FDA adverse drug reports (ADR) database for up to the first five years of actual sales for ~335 drugs approved for marketing between 1978 and 1997. The dataset was then limited to drugs that had at least 5 reports for any one of the adverse events and adverse events that had at least 5 reports for any one drug (i.e., an average of one report per year). The result was a matrix of 262 drugs *versus* 114 neurologically related adverse events. Hierarchical cluster analysis of the dataset placed ~20% of the drugs into clusters with correlation coefficients of greater than 0.9. Half of those drugs were found in clusters containing other drugs with similar clinical indications, demonstrating the ability of this approach to detect relationships between drugs. Clusters containing drugs with dissimilar indications may be related by other activities dependent on common structural components. Next we looked at how the adverse event categories related. Cluster analysis placed ~25% of the adverse events into clusters with correlation coefficients of greater than 0.9. The National Library of Medicine's MeSH (medical subject heading) system was used to evaluate the relationships of the adverse event clusters. Half of the highly correlative events were found in clusters with similar MeSH headings. Combining data for adverse events with similar profiles creates composite event categories that may increase their utility in developing structural alerts. This database combined with structural data for this set of drugs has been used to develop neurotoxicity modules for a quantitative structure-activity relationship expert system.

780 CONTRIBUTION OF INTERLEUKIN-10 (IL-10) TO LANGERHANS CELLS MIGRATION IN MICE PROVOKED BY CHEMICAL CONTACT AND RESPIRATORY ALLERGENS.

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Following prolonged topical exposure of BALB/c strain mice to chemical contact and respiratory allergens divergent immune responses indicative of preferential T helper (Th)1- and Th2-cell activation are elicited, respectively. Thus, under conditions of exposure that induce equivalent levels of immunogenicity with respect to lymph node cell (LNC) proliferation and IgG antibody production, only respiratory allergens provoke IgE antibody and a Th2 cytokine secretion profile by LNC.

We have now investigated the contribution of dendritic cells (DC) to the development of these polarised immune responses. We report that topical exposure of mice to contact allergens such as 2, 4-dinitrochlorobenzene (DNCB; 1%) and oxazolone (Ox; 0.5%) results in a significant reduction in epidermal Langerhans cells (LC) frequency (20-30%) within 4 hours. In contrast, similar treatment with the respiratory allergen trimellitic anhydride (TMA; 25%) failed to provoke such changes in LC densities after 4 hours, although, LC values were significantly reduced by 17 hours. Enumeration of DC accumulating in draining lymph nodes (DLN) 18 hours following treatment with these chemicals revealed that increases in DC numbers induced by DNCB and Ox were 2-fold greater than those associated with exposure to TMA. Similar DC frequencies to those recorded for TMA were provoked by topical treatment with fluorescein isothiocyanate (FITC; 1%), a sensitizing chemical which also induces a Th2-type immune response. Furthermore, following local administration of a neutralizing anti-IL-10 antibody prior to exposure at the same site to chemical, TMA- and FITC- induced DLN DC accumulation was increased significantly, whereas responses to Ox and DNCB were unaffected. These data suggest that chemical contact and respiratory allergens display differential kinetics of LC migration and DC accumulation and that the delayed response observed to those chemicals that induce Th2-type responses may be due to an early inhibitory signal provided by IL-10.

781 MURINE cDNA ARRAY EXPRESSION PROFILING OF CUTANEOUS SULFUR MUSTARD INJURY.

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The chemical warfare agent, sulfur mustard (SM) produces skin blister formation with a severe inflammatory reaction in exposed individuals. The mechanism of SM induced tissue injury is not well understood and there is no established medical treatment to counter its toxicity. Our previous studies identified increases in inflammatory gene expression in both *in vivo* and *in vitro* models of vesicant injury. This study examined SM-induced alterations in gene expression in mouse skin using cDNA arrays to identify transcriptional events associated with SM toxicity. Ear skin from 5 mice, paired as SM-exposed (right ear) and unexposed (left ear) from the same animal, at seven exposure doses (5, 10, 20, 40, 60, 80, and 160 µg) was harvested at 24 hours post-exposure. The highest SM dose (160 µg) produced severe injury characterized by edema, dermal infiltration of inflammatory cells, premature death of basal layer epidermal cells, and epidermal-dermal separation. The lowest concentration (5 µg) produced no apparent tissue damage. Screening of 1176 cDNAs that were normalized as exposed/unexposed skin samples from the same mouse identified numerous genes activated or repressed ≧2-fold, with the number of altered genes increasing with SM dose. The mean level of expression (5 animals) for 54 genes increased and 78 genes decreased ≧2-fold at one or more exposure doses when compared to the 5 µg dose. Real-time RT-PCR confirmed the up-regulation of IL-1β and IL-6. The transcriptional activation or repression of a number of genes not previously known to be associated with SM toxicity was identified. (Supported by the US Army Medical Research and Materiel Command under Contract No. DAMD17-99-D-0010).

782 DERMAL PENETRATION OF TESTOSTERONE *IN VIVO*: PREDICTION FROM SKIN *IN VITRO*.

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Testosterone, the steroid hormone, has been used in topical applications to achieve a systemic effect. Due to its lipophilicity (Pow 3.32) it is efficiently absorbed and it has recently been proposed as a marker chemical for predictions of percutaneous penetration using skin *in vitro*. However there is limited data on direct comparisons of equivalent applications *in vivo* and *in vitro*. Whole or dermatomed (230 µm) fresh skin from 28 day old male Wistar rats was mounted in Scott-Dick flow-through diffusion cells at 32°C for 24 hrs. Receptor medium (Eagle's Minimal Essential Medium [MEM] supplemented with 2% (w/v) BSA, pH 7.4) was pumped below the skin at 1.5 $ml h^{-1}$. [4-^{14}C] Testosterone (10 µl [17 kBq]) was applied to the skin surface (0.64 cm^2) as 200 $\mu g ml^{-1}$ in ethanol and receptor fluid samples were collected hourly for 24 hrs. In a parallel *in vivo* study in four rats 100 µl testosterone was applied to a shaved dorsal area (6.25 cm^2) at 200 $\mu g ml^{-1}$. Lomir dermal jackets protected the backs of the animals for a 24 hr period. Animals were individually housed in metabolism cages from which the excreta are collected. Material in the carcass, urine and faeces was determined. *in vivo* 12% of the dose was recovered from the skin surface, 2% within the stratum corneum, 5% within the skin at the application site, 18% in urine, 2% in faeces and 40% in carcass. Therefore 65% of the dose was absorbed beyond the stratum corneum. By comparison, *in vitro* data indicated that 35% of the applied dose remained unabsorbed on the skin surface, 3% within the stratum corneum, 7% (1% if dermatomed skin) within the skin and 30% (40% if dermatomed skin) was recovered in the receptor medium. In conclusion *in vivo* absorption of testosterone in the rat can be reasonably predicted by an *in vitro* model however the underestimation may arise due to limited perfusion of the skin which cannot be modeled adequately . (Supported by SEAC Toxicology Unit, Unilever Research.)

783 NOVEL PEPTIDES PROTECT AGAINST SULFUR MUSTARD-INDUCED SKIN TOXICITY.

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Our previous studies demonstrated the protective effect of topical iodine treatment against sulfur mustard (SM)-induced skin lesions. It was proposed that the mechanism involves iodine-induced production of skin protective factors. To test this hypothesis guinea pig skin was exposed to heat followed by treatment with iodine and skin extraction. Intradermal injection of the tissue extract resulted in statistically significant reduction of 69% in SM-induced ulceration area. Two pharmacologically active peptides were identified, partial sequence of histone H2A and fibrinopeptide A; administration of each of these peptides resulted in statistically significant reduction of 67% and 62% respectively, in SM-induced ulceration area. The peptides were coadministered with a cocktail of proteinase inhibitors to prevent degradation. In order to stabilize the peptides we prepared a series of N-methylated analogs in which the hydrogen atom of the nitrogen of the peptide bond was substituted by a methyl group. The most active N-methylated analog of the partial sequence of histone H2A caused statistically significant reduction of 81% in SM-induced ulceration area in the absence of proteinase inhibitors. The present study proposes a new pharmacological approach for whole skin surface protection, and maybe for whole body protection, against vesicants and other noxious stimuli (supported by USAMRMC Cooperative Agreement No. DAMD17-98-2-8009).

784 UPTAKE AND ACTIVITY OF CAPSAICIN AND NONIVAMIDE IN HUMAN SKIN AS A FUNCTION OF VEHICLE.

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Capsaicin and its analog, nonivamide, are commonly used in the preparation of commercial "pepper sprays" for use as less-than-lethal deterrents. These natural products produce a skin erythema response that can be quantified using the a* scale of the L*a*b* uniform color space of a reflectance colorimeter. This study compared erythema with the uptake of capsaicinoids and nonivamide in ventral forearm stratum corneum in seven subjects (47.3 ± 15.7 yrs) as a function of the vehicle: 95/5 (v/v) ethanol/methanol (EtOH), 70/30 (v/v) isopropyl alcohol/water (IPA), 80/20 (v/v) mineral oil/isopropyl alcohol (MO) and 80/20 (v/v) propylene glycol/isopropyl alcohol (PG). Solutions were prepared using 30 mcg/mL of capsaicin or nonivamide in the above vehicles and applied as a single, unoccluded 5 mcL dose to both ventral forearms of each subject for 10 minutes in a randomized manner. Capsaicinoid concentration was quantified by harvesting stratum corneum from treated sites using twenty 1.3 cm diameter adhesive discs that were subsequently weighed, extracted and analyzed for capsaicinoids using LC-MS. Erythema was quantified over 90 minutes after residual product removal on the other arm with a Minolta CR200 colorimeter. The amount of stratum corneum weight harvested from the various sites was independent of the vehicle applied (~ 264 mcg). The first ten adhesive discs contained the majority of capsaicinoids and nonivamide in the skin: EtOH and IPA delivered 3-5X greater amount of capsaicinoids to the stratum corneum than MO or PG ($p < 0.001$), while EtOH delivered 2-5X greater amount of nonivamide than IPA, MO or PG ($p < 0.05$). EtOH delivered 2X more nonivamide than capsaicinoids into stratum corneum, yet area-under-the a* scale-time curve (AUEC a*) demonstrated similar erythema independent of vehicle. Relative potency (AUEC a*/concentration) of capsaicinoids was 2X greater than nonivamide in EtOH and IPA vehicles, but similar in PG and MO. Thus, vehicle influences both delivery and potency of capsaicin and nonivamide in human skin *in vivo*.

785 TRANSCRIPTOMIC PROFILES IN RAT SKIN FOLLOWING DERMAL EXPOSURE TO SODIUM LAURYL SULFATE.

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Dermal irritation continues to be an important yet poorly understood occupational health issue. Irritant contact dermatitis (ICD), the most common occupational skin disease, costs government and industry millions of dollars each year. Gene array

technology can serve as a tool for assessing chemical irritancy potential. To determine the transcriptional response to dermal irritants, male Fisher 344 rat skins were exposed to 10% sodium lauryl sulfate (SLS, a model skin irritant) for 1 h *in vivo*. Skin total RNA was isolated at 0 h (control), and 1 and 4 hrs following the beginning of the exposure to monitor transciptomic profiles using the Affymetrix RatTox U34 array. Our results indicated that dermal exposure to SLS resulted in a significant change of gene expressions: more than 20 genes (ie., inflammatory, Bcl-2-related, oncogenes) were increased >2-fold, whereas more than 24 genes (ie., cytochrome P450-related, tumor suppressor) showed a >2-fold decrease (1h vs 0 h controls). By 4 hr, more than 60 genes (ie., inflammatory, oxidative and cellular stress, cell cycle, transcription factors, Bcl-2-related) were up-regulated and more than 10 genes (transcription factors, junctional proteins) were down-regulated by a factor >2-fold when compared to the 0 h controls. A strong temporal change of gene expression was observed in the skin following exposure to SLS. Characterization and analysis of the transcriptomic response of skin to SLS can enhance the risk assessment of dermal irritants.

786 *IN VITRO* ASSESSMENT OF OXIDATIVE STRESS AND CYTOTOXICITY IN LIVING DERMAL EQUIVALENTS EXPOSED TO M-XYLENE.

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Dermal exposure to volatile organic chemicals (VOCs) can lead to irritation, inflammation, and cytotoxicity *in vivo*. Using an improved *in vitro* exposure method for VOCs, we wanted to assess oxidative stress and cytotoxicity in dermal fibroblasts exposed to m-xylene. Dermal equivalents containing $2.5x10^5$ cells were incubated for 1 or 4 hr in a m-xylene/culture medium mixture (range=0.5 to 11.5 µg/ml m-xylene). Following exposure, cell viability (MTT assay and cellular LDH activity), cellular thiol levels, and endogenous catalase activity were measured. At 1 and 4 hr, cell viability decreased with increasing m-xylene concentration. The EC_{50} calculated using the MTT assay were 8.33 ± 0.26 and 6.85 ± 0.33 µg m-xylene/g tissue at 1 and 4 hr, respectively. The EC_{50} at 4 hr was significantly lower ($P<0.05$) compared to the EC_{50} calculated from the 1 hr exposures. At 1 hr, the EC_{50} determined from measuring cellular LDH activity was 23.7 ± 0.88 µg m-xylene/g tissue. By 4 hr, the EC_{50} had decreased significantly ($P<0.05$) to 9.24 ± 0.05 µg m-xylene/g tissue. A temporal decrease in the levels of endogenous antioxidants (catalase and thiols) was also observed with increasing m-xylene concentration. Pretreatment of dermal equivalents with the antioxidant N-acetylcysteine significantly increased ($P<0.05$) cell viability. These results suggest oxidative stress may promote m-xylene-induced cytotoxicity in living dermal equivalents. Moreover, these VOC-induced cellular responses could ultimately be related to actual dermal exposure scenarios. (Supported by NIOSH/CDC and AFOSR/NL)

787 PREDICTION OF MOLECULAR MECHANISM OF SKIN IRRITATION AFTER ACUTE EXPOSURE TO SODIUM LAURYL SULFATE.

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Sodium lauryl sulfate (SLS) is an anionic surfactant, causing irritant contact dermatitis. Skin irritation by this detergent is not well understood due to the complex molecular interactions. Knowledge of molecular mechanisms and the relationship between duration of surfactant exposures on the skin and the degree of irritation is limited. Here we measured an early inflammatory mediators, interleukin 1-alpha (IL-1α), inducible nitric oxide synthase (iNOS), nitric oxide (NO) and ROS in response to two different concentrations of SLS (1% and 10%)-induced irritation.The dorsal thoracic aspects of male F-344 rats were exposed to SLS for 1 hr using Hill Top Chambers. At 0, 1, 2, 4 and 6 hours after exposure, skin samples were processed for analysis. Western blot of 1% SLS exposed skin samples showed an increase in IL-1α protein levels from 13-37% over controls at various time points, while IL-1α in 10% SLS samples only increased by 10%. This protein induction reached a maximum at 2 hr. The change in iNOS levels in 1% SLS skin over the respective controls was comparable to change in 10% SLS exposed skin samples but occurred at 4 hours. However, NO levels showed a different response. Like NOS protein level in 1% SLS exposed samples, NO level increases peaked at 4 hr after beginning of exposure. In 10% exposed samples NO level peaked at 1 hr after exposure and gradually decreased. There was a significant generation of ROS seen in 1% SLS exposed skin, which reflects the damaging effects on the skin. The reduced level of thiol further strengthens the potential association of oxidative stress and skin damage during SLS exposures. These results suggest that the change in the levels of skin's molecular and biological response to SLS exposure explained the biochemical mechanisms associated with SLS-induced irritation (Supported by CDC/NIOSH RO1 OH03654-03).

788 HISTOPATHOLOGIC ASSESSMENT OF ACUTE DERMAL EXPOSURE TO *M*-XYLENE, *D*-LIMONENE AND SODIUM LAURYL SULFATE IN RATS.

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Skin exposure to organic chemicals and solvents may cause skin irritation and generate proinflammatory mediators that initiate an irritant response. Histopathology can serve as a fixed reference point in a changing microenvironment for explanation of concurrent cellular responses. Changes in the levels of selected proteins in the irritant cascade were quantified after an exposure and compared to visible tissue changes. Here we describe the dermal pathology of male F-344 rats exposed to meta-xylene, *d*-limonene and sodium lauryl sulfate for one hour using Hill Top Chambers®. Skin samples were collected at zero, one, two, four and six hours after the end of the exposure. Light microscopic evaluation was performed on formalin-fixed and paraffin embedded skin sections. Hematoxylin and eosin stained sections were assessed and scored. A single topical exposure to meta-xylene, *d*-limonene, and sodium lauryl sulfate resulted in granulocyte infiltration in rats as early as 2 hours. Segmental detachment of the epidermis from the dermal interface occurred in prolonged exposures of meta-xylene and *d*-limonene compared to the control groups. These findings were interpreted to be the consequences of potential up-regulation of inflammatory mediators that result in skin damage. (Supported by CDC/NIOSH RO1 OH03654-03)

789 PYRIDOSTIGMINE BROMIDE SUPPRESSES IL-8 IN HUMAN EPIDERMAL KERATINOCYTES AND IN ISOLATED PERFUSED PORCINE SKIN EXPOSED TO DEET AND PERMETHRIN.

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Gulf War personnel were given pyridostigmine bromide (PB) as a prophylactic treatment against possible organophosphate exposure and then exposed to the insecticide permethrin (Pe) and the insect repellent DEET. Simultaneous exposure and the interaction of these compounds could contribute to the ill health effects in the Gulf War veterans. The purpose of this study was to assess the effects of DEET and /or systemic PB on permethrin absorption. A large combination of topical mixtures of permethrin and/or DEET in various vehicles were applied to isolated perfused porcine skin flaps (IPPSFs). The absorption data has been reported elsewhere. Concentrations of interleukin-8 (IL-8) and TNF-α were assayed in the absorption studies to probe for a potential inflammatory effects after complex mixture application. IPPSF's (n=4/treatment) were topically dosed with mixtures of Pe, DEET, Pe/DEET, and ethanol (EtOH). Each treatment was repeated with perfusate spiked with 50 ng/ml of PB. Timed IPPSF venous effluent samples (0, 0.5, 1, 2, 4, and 8 hrs) were assayed by ELISA for IL-8 and TNF-α and by EIA for PGE_2. Surprisingly, across most topical mixture combinations, release of IL-8 and TNF-α were significantly suppressed when PB was infused into the IPPSFs. To probe the potential mechanism of this PB effect, human epidermal keratinocyte (HEK) cell cultures were exposed to Pe, DEET, Pe/DEET, with and without PB in DMSO vehicle. IL-8 was assayed at 1, 2, 4, 8, 12, and 24hrs. PB suppressed IL-8 in permethrin treatments from 4 to 24 hrs supporting the IPPSF results. In conclusion, these studies clearly demonstrate that systemic exposure to PB suppressed the cytokine IL-8 release from keratinocytes. The relation of this finding to PB-induced changes in permethrin absorption and/or other factors in the pathogenesis of Gulf War illness deserves further attention. (Supported by USAMRMC Grant DAMD-17-99C-9047)

790 LACK OF EFFECTS OF SULFUR MUSTARD AND JP-8 JET FUEL ON PERCUTANEOUS ABSORPTION OF SIMULTANEOUSLY ADMINISTERED TOPICAL PERMETHRIN AND DEET.

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Systemic absorption of topically-applied permethrin and/or DEET have often been cited as potential toxicants involved in the etiology of the Gulf War illness. Previous studies in our laboratory have implicated pyridostigmine bromide (PB) as a potential modulating factor on permethrin dermal absorption. Others have postulated a toxicodynamic interaction with systemically absorbed permethrin and DEET. The purpose of the present study was to assess the effects of simultaneously administered JP-8 jet fuel or low-level (sub-blistering) sulfur mustard (HD) on permethrin

and DEET percutaneous absorption using the isolated perfused porcine skin flap (IPPSF) model. IPPSFs were topically dosed with 40 μg/cm^2 ^{14}C permethrin and/or 75% DEET in various ethanolic vehicles with or without HD (40 μg/cm^2) or 75% JP-8. A total of 40 IPPSFs were studied (n=4 per treatment combination). Permethrin absorption was assessed by measuring radioactivity in perfusate and skin after completion of 8 hour experiments. DEET absorption was assessed by HPLC. Consistent with previous data, PB tended to enhance permethrin absorption, detected as ^{14}C radioactivity. Co-administration of JP-8 slightly enhanced permethrin absorption, however not to the extent that was seen with PB. DEET absorption was not effected. HD had no consistent effect on either permethrin nor DEET absorption or skin penetration. These results suggest that neither exposure to JP-8 nor low level HD significantly increases percutaneous absorption of either topically applied permethrin or DEET, and thus cannot be viewed as an additional risk factor for any role that these topically applied chemicals might play in Gulf War illness. (Supported by USAMRMC Grant DAMD-17-99C-9047).

791 ASSESSMENT OF CYTOTOXICITY TO HUMAN EPIDERMAL KERATINOCYTES AFTER EXPOSURE TO ALIPHATIC AND AROMATIC HYDROCARBONS.

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Our laboratory has shown that jet fuels cause dermal toxicity and the release of the proinflammatory cytokine IL-8 from human epidermal keratinocytes (HEK). More than 220 aliphatic and aromatic hydrocarbons are commonly formulated in jet fuels but the cytotoxic effects of the individual hydrocarbon are unclear. One major problem being that vehicle effects often confound chemical toxicity studies. The purpose of this study was to assess the dermal toxicity induced by each jet fuel hydrocarbon using a single vehicle. Eight aliphatic (dodecane, undecane, tridecane and hexadecane) and aromatic (benzene, toluene, xylene and napthalene) hydrocarbons at various concentrations in mineral oil were dosed directly on HEK. Cell viability at 24 hrs and IL-8 release from HEK at 4, 8, 12 and 24 hrs were assayed. There was no significant decrease in cell viability by all hydrocarbons. However, there appears to be significant differences among each aliphatic and aromatic hydrocarbon with respect to their effects on IL-8 release. Not all HEK exposed to hydrocarbons at normal jet fuel concentrations of 0.5% to 5% increased IL-8 production. Exposure to mineral oil alone for 30 min did not affect cell viability or IL-8 release. In conclusion, we have demonstrated that mineral oil is a suitable vehicle for studies in HEK culture systems especially for high-dose, short-term exposures of compounds with a wide range of lipophilicity. Utilizing 96-well plates also allows for the evaluation of cytotoxic effects of individual and clusters of hydrocarbons to be screened in a relatively short period of time. (US Air Force Office of Scientific Research F49620-01-1-0080)

792 DERMAL ABSORPTION OF AVERMECTIN: FORMULATION AND SPECIES DIFFERENCES.

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Avermectins are approved for topical application in cattle only. There is however some concern that topical application in other domestic animals, may result in significant dermal absorption and possible violative residues in animal-derived food products. The primary aim of this study was to assess dermal disposition of [^{3}H]-avermectin in skin from food-producing species *in vitro* using commercial alcohol-based and oil-based formulations. Skin sections from cattle, sheep, goats, and pigs, were perfused in a flow-through diffusion cell system for 8 hours. Skin sections were dosed with 150 μg/cm^2 of ^{3}H-avermectin in 20 μl of 75% isopropanol+25% crodemol, 100% mineral oil, or 100% isopropyl alcohol. Perfusate samples were collected at various time points and surface swabs and dose skin samples were obtained at 8 hours. ^{3}H-avermectin absorption ranged from 0.09 - 0.20 % dose and there were no significant differences between formulations for ^{3}H-avermectin absorption in each species. The presence of isopropyl alcohol significantly increased skin deposition in all species when compared the oil formulation. Absorption was significantly greater in cattle skin than in pig skin for the isopropyl alcohol formulations, but there were no significant species differences for the oil formulation. While 11.69 - 50.23 % dose remained on the skin across species, the highest skin concentrations were in goat skin (28.09% dose) and the lowest skin concentrations were in pig skin (1.50% dose). In summary, these 8-hour preliminary experiments demonstrated that the alcohol-based formulations compared to oil-based formulations enhanced ^{3}H-avermectin absorption and skin deposition in several animal species, and that this effect is more likely to be observed in ruminant species than in porcine species. (Supported by North Carolina State University Grant FR&PD)

793 CUTTING FLUID FORMULATIONS INFLUENCE THE DERMAL DISPOSITION OF LINEAR ALKYLBENZENE SULFONATE (LAS).

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Linear alkylbenzene sulfonate (LAS) is often added as a surfactant to cutting fluid formulations to enhance the performance of metal machining operations. Unfortunately, LAS and other cutting fluid additives can cause contact dermatitis in workers in the metalworking industry. The purpose of this study was to assess membrane absorption and deposition of ^{14}C-LAS when topically applied to inert membranes (silastic membranes) and porcine skin in *in vitro* flow-through diffusion cell system as mineral oil or polyethylene glycol (PEG) mixtures. ^{14}C-LAS mixtures were formulated with 3 other additives; namely, 0 or 2% triazine (TRI), 0 or 5% triethanolamine (TRE), and 0 or 5% sulfurized ricinoleic acid (SRA) as follows: TRI, TEA, SRA, TRI+TEA, TRI+SRA, TEA+SRA, TRI+TEA+SRA. In silastic membranes, LAS absorption ranged from 0.09 - 0.19% dose, and there were no differences between corresponding mineral oil and PEG mixtures. Membrane levels were greatest with TRI only in mineral oil and PEG mixtures. In porcine skin, ^{14}C-LAS absorption ranged from 0.06 - 0.32% dose, and there were significant differences between several mineral oil and PEG mixtures. LAS penetration into stratum coneum (SC) was often greater in mineral oil than in PEG mixtures. Surprisingly, LAS absorption was significantly greater in pig skin than in silastic membranes for PEG mixtures containing TRI+TEA. These observations suggest that although very little LAS is absorbed, cutting fluid components can alter LAS deposition into the SC and skin. Furthermore, chemical-biological interactions in viable skin with synergism with a biocide (TRI) and an amine (TEA) may be important determinants for LAS disposition in skin. (Supported by NIOSH Grant R01-OH-03669)

794 BIOMECHANICAL MONITORING TECHNIQUES TO ASSESS SULFUR MUSTARD LESIONS IN WEANLING PIGS.

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Sponsor: C. Olson.

The chemical warfare agent, sulfur mustard (SM) produces severe skin injury and there is no established medical treatment. In the present study, we investigated the use of biomechanical measurements as objective, quantitative, and non-invasive means for conducting SM burn assessments to evaluate medical treatments. Additionally, assessments were confirmed by histopathological evaluation. Six animals per group were exposed to SM (2- or 30-min exposure to 400 μL SM applied to each of 6 abdominal sites) and six animals received sham (control; 400 μL deionized water for 30-min) exposures. Each site was evaluated on days 0 and 2. Two-min and 30-min groups were significantly different from control and significantly different from each other, as characterized by histopathological endpoints of burn depth, basal cell necrosis, depth of necrosis, and vascular necrosis. Two-min and 30-min dermal burns were significantly different from control using redness (Chroma Meter) and transepidermal water loss (Evaporimeter), but not significantly different from each other. The 2-min burns and control were significantly different from 30-min burns for skin thickness (Ultrasound). Compared to control, the 2-min burns were significantly increased and the 30-min burns were somewhat decreased for microcirculation blood flux (Laser Doppler). We demonstrate that both biomechanical and histopathological evaluations are useful methods for characterizing SM burns in a weanling pig model. (Supported by the US Army Medical Research and Materiel Command under Contract No. DAMD17-89-C-9050.)

795 α-AMYLCINNAMALDEHYDE (α-ACA) AND α-HEXYLCINNAMALDEHYDE (α-HCA) DO NOT PRODUCE DERMAL SENSITIZATION OR CROSS-SENSITIZATION IN HUMANS.

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Both α-ACA (heptanal, 2-(phenylmethylene)-) and α-HCA (octanal, 2-(phenylmethylene)-) are important fragrance ingredients. Studies were conducted to evaluate the potential of these two materials to induce sensitization and cross-sensitization in a normal human population. Sensitization was evaluated using either a modified Draize human repeated insult patch test (HRIPT) procedure consisting of nine 24 hour occluded induction applications followed two weeks later by a 24 hour occluded challenge application; or by a human maximization test procedure consisting of occluded induction applications to the same site for five alternate-day 48 hour periods followed ten to fourteen days later with a 48 hour occluded challenge application. Cross-sensitization between these two materials was also evaluated using a HRIPT procedure. α-ACA produced no sensitization in 71 human

volunteers when tested at 6% in human maximization tests or in 95 human volunteers when tested at 20% in a HRIPT. α-HCA produced no sensitization in 81 human volunteers when tested at 12% in human maximization tests or in 324 human volunteers when tested in HRIPTs at dose levels ranging from 5% to 20%. Cross-sensitization was not observed in 95 subjects tested with 20% α-ACA and then cross-challenged with 20% α-HCA or in 95 subjects tested with 20% α-HCA and then cross-challenged with 20% α-ACA. Although sensitization has been reported in animal tests, the results of these human studies indicate no evidence of dermal sensitization with α-ACA or α-HCA after repeated application. Additionally, no evidence of cross-sensitization was produced with either substance.

796 A NOVEL TECHNIQUE TO STUDY PERCUTANEOUS ABSORPTION BY USING A SILASTIC MEMBRANE COATED FIBER.

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Silastic membrane coated on a section of fused silica fiber has been used successfully as an analytical tool to extract analytes from aqueous solution. In this study, the silastic membrane coated fiber was used as a permeation membrane to study the percutaneous absorption of model pesticides. It represents a half compartment of the conventional Franz diffusion cell, which allows for more detailed permeation kinetics to be investigated. The membrane coated fiber was immersed in the magnetic stirred donor phase to adsorb the permeants from the solution, the fiber was then transferred directly into the injection port of a Gas Chromatograph/Mass Spectrometer for quantitative analysis. This technique can assess the permeation amounts at various permeation times, stirring speeds and donor compositions. A theoretical model was proposed to describe the permeation processes of the silastic membrane coated fiber in terms of diffusion parameters. This model allows for the boundary layer between the donor phase and the silastic membrane to be considered, it's thickness is assumed to be constant under steady-state diffusion. The permeation kinetics of 30 different compounds having a wide range of partition coefficients were examined utilizing a silastic membrane coated fiber. The experimental permeation flux and time profiles were well described by the proposed mathematical model. The thickness of the boundary layer, the diffusion coefficients in the donor phase and in the silastic membrane, and the partition coefficients can be measured simultaneously with this novel technique. This approach would foster development of high-throughput determination of skin diffusion parameters. (Supported by NIOSH R01-OH 03669 and 07555)

797 A GENOMICS APPROACH FOR THE DEVELOPMENT OF AN *IN VITRO* METHOD FOR SKIN SENSITIZATION.

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For the development of an *in vitro* method for predictive skin sensitization testing, a genomics approach is being used to elucidate pathways that are involved in the immune recognition of chemical allergens by dendritic cells (DC) and T-cells, the key cells involved in the induction and elicitation of allergic contact dermatitis. The overall aims are to identify and characterize those genes in both DC and T cells which provide sensitive, selective and robust markers of skin sensitization and which allow discrimination between contact allergens and skin irritants. To date, there are no published reports describing genomic-scale analysis of the early changes induced in either T cells or DC resulting from antigen-specific interactions between the two cell types. There are a limited number of reports regarding microarray analysis of T cell activation following heat shock or exposure to activating agents such as staphylococcal enterotoxin B or PHA and phorbol ester. While changes in the expression of some genes were found to be similar in all activated T cells, there were some changes that were dependent upon the nature of the activating signal. Therefore, to gain an understanding of the changes in gene expression that are induced by antigen-specific T-cell/DC interactions in humans, we examined T cell activation *via* the T cell receptor, using anti-CD3, with co-stimulation provided by DC. An *in vitro* co-culture system comprised of peripheral blood derived DC as antigen presenting cells and responding T cells was used. Total RNA was isolated from co-cultures after 2 and 6 hrs of culture with and without anti-CD3. Genomics analysis was conducted using the Affymetrix GeneChip® human U95Av2 array. Changes in gene expression of 2-fold or more were observed in 108 genes, ranging from an up-regulation of 42-fold to down-regulation of 10-fold. Information gained in this study will be used as a benchmark in the examination of primary responses in non-sensitised T cells co-cultured with hapten-treated DC.

798 INHIBITION OF PHOTOCARCINOGENESIS BY TOPICAL ADMINISTRATION OF DAPSONE.

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Dapsone (DAP) is a potent anti-inflammatory and anti-bacterial approved as an oral therapy for leprosy and dermatitis herpetiformis. While effective for these diseases and acne, the relatively high oral doses of DAP can cause adverse events. Topical administration would deliver high local DAP concentrations to skin but greatly reduce the systemic dose and side effects. Because of the high absorbance of DAP in the ultraviolet portion of the solar spectrum and it's anticipated chronic topical administration to facial skin exposed to sunlight, a photocarcinogenesis test assessed the potential of topical DAP to modify the development of solar-simulated radiation (SSR)-induced skin tumors in Crl:SKH1-hrBR hairless mice. Dapsone was formulated in a diethyl glycol monoether (DGME)-based vehicle at DAP/DGME ratios of 1%/10%, 3%/17.5% or 5/25%. The study design included a group of mice administered vehicle (25% DGME) and low and high UVR calibration groups. Mice were administered formulations and exposed to SSR five days per week for 40 weeks then observed for an additional 12 weeks. Clinical observations and tumor mapping were performed weekly. Cutaneous responses indicated DAP administration reduced the incidence of erythema, edema and thickening in the groups of mice administered the DAP/DGME formulations as compared with mice exposed to SSR alone. Dapsone concentration-dependent hyperactivity occurred throughout the 40 week dosing period. The unbiased median latent weeks to tumor, sexes combined, were 41.50, 47.50, 48.00 and 49.00 weeks for the vehicle, low, middle and high dose DAP/DGME formulations, respectively, compared to 41.50 weeks for the low UVR calibration group, indicating a delay in tumor development in the DAP/DGME formulation-treated groups and no vehicle effect. In conclusion, topical DAP administration does not enhance photocarcinogenesis in the hairless mouse model and indeed affords protection against SSR-induced skin tumor development and cutaneous responses to SSR exposure.

799 ABSORPTION OF ^{14}C-CYLOTRIMETHLYENETRINITRAMINE (RDX) THROUGH HUMAN SKIN *IN VITRO*.

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Dermal exposure of the explosive chemical RDX can occur to humans during the manufacturing process and occupational (load, assembly and package) operations. There is no information on dermal absorption of RDX in humans for risk assessment. We studied dermal absorption of RDX in human skin *in vitro* in flow-through diffusion cells. RDX (38.46μg / 0.5 μCi) in acetone (10 μl) was applied to the skin and collected as diffused receptor fluid for every 6 hr up to 24 hrs. At the end of the experiment, the unabsorbed RDX was washed with soap water and water with cotton swabs, and radioactivity present in washings was determined. The RDX absorbed or penetrated in the skin was also determined by separating stratum corneum, epidermis and dermis at the end of the experiments. Our results show that the total of approximately 6 % of applied dose was absorbed in the skin (receptor fluid and skin) in 24 hr. The absorption of RDX in the receptor fluid was relatively slow with about 1.28 % of applied dose in 24 hrs. Preliminary results of analysis of stratum corneum (tape strips), epidermis and dermis revealed that a majority of radioactivity localized in the upper stratum corneum and epidermis and it was very low in the dermis of the skin after 24 hrs. The total recovery of applied dose (receptor fluid, skin and washings) was about 80 %. These results show that RDX is absorbed in the skin but diffusion through skin was relatively slow. This may be due to liposolubility of RDX in the skin. The estimated levels of RDX in the skin can be used to evaluate health risks associated with dermal exposure (Abstract does not reflect US Army policy).

800 *IN VITRO* HUMAN SKIN PENETRATION OF THE RADIOLABELLED FRAGRANCE MATERIALS, AHTN AND HHCB.

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in vitro human skin permeation rate and distribution of two radiolabelled polycyclic musks, AHTN and HHCB, following application under non-occlusive conditions was determined. The studies followed the European Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) guidelines and used human cosmetic reduction skin from surgery. Screening studies were conducted to identify the most appropriate receptor fluid, which for these studies was 6% of the surfactant VolpoN20 in physiologically balanced saline. The skin samples were heat separated and the epidermal membranes comprising both the stratum corneum and the epidermis were used. Because the system was static, the dermis was stripped

away. The integrity of the membranes was determined prior to dosing using tritiated water. A 1% solution of radiolabelled (^{14}C) test material in ethanol was applied to the membrane (20 µliters/cm^2). After 24 hours, the amount of material in the receptor fluid was determined using a scintillation counter. Mass balance was determined and included test material collected from the surface wipes, 10 skin strippings, remaining epidermis, receptor phase, donor chamber, and filter paper supports. After 24 hours, 0.38% of the applied AHTN dose permeated the epidermal membranes into the receptor phase. The total absorbed dose for AHTN, determined by adding the amounts of test material in the remaining epidermis, the receptor phase and the filter paper was 4.1% of the applied dose. The evaporative loss was 2.9%. Overall recovery of applied AHTN was high at 92.5% or 95.4% when loss due to evaporation is included. For HHCB, the penetration through the epidermal membranes was 0.40% after 24 hrs. The total absorbed dose for HHCB was 5.2% of the applied dose. The evaporative loss was 2.4%. Overall recovery of applied HHCB was good at 92.1% or 94.5% with evaporative loss included. The results of these studies show that the percutaneous absorption of both of these fragrance materials was low - 4.1% for AHTN and 5.2% for HHCB.

801 *IN VITRO* HUMAN SKIN PENETRATION OF SEVEN RADIOLABELLED FRAGRANCE MATERIALS.

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in vitro human skin penetration studies on 7 fragrance materials were conducted according to the FDA guidelines. The materials studied were acetyl cedrene, estragole, methyl atrarate, methyl dihydrojasmonate, methyl eugenol, α-methyl-3, 4-methylenedioxy hydrocinnamic aldehyde (MMDHCA) and 1, 2, 3, 4, 5, 6, 7, 8-octahydro-2, 3, 8, 8-tetramethyl-2-acetonaphthone (OTNE). Each of the test materials was radiolabelled with ^{14}C. The full thickness human skin samples were either from the breast or abdomen of cosmetic surgery patients. The skin samples were heat separated and the epidermal membranes were used in the studies. The epidermal membranes were mounted on filter paper supports. The supports, with the membranes, were then placed onto diffusion cells and trimmed to size. The integrities of the membranes were determined using tritiated water. Twenty µl of a 1% solution of each material in ethanol was applied to the surface of the membranes. At 2, 8, 24, 36, and 48 hours 200 µl samples of the receptor fluid (50% ethanol/water solution) were taken from the receptor chamber and analyzed by liquid scintillation chromatography. Evaporative loss of each fragrance material was also determined. At 24 and 48 hours the following percentages of the applied dose permeated into the receptor phase: 6 and 11% of acetyl cedrene, 17 and 16% of estragole, 14 and 20% of methyl atrarate, 31 and 46% of methyl dihydrojasmonate, 36 and 34% of methyl eugenol, 42 and 50% of MMDHCA, and 9 and 15% of OTNE. These results suggest varying percutaneous absorption of these materials. However, it should be noted that the donor phase became somewhat depleted through evaporation and could affect the amount of test material available for absorption.

802 INVESTIGATION OF THE SENSITIZATION POTENTIAL OF MUSK KETONE USING THE GUINEA PIG MAXIMIZATION TEST (GPMT).

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As part of a risk assessment on musk ketone, a widely used synthetic musk fragrance ingredient, a Guinea Pig Maximization Test (GPMT) was used to investigate the sensitization potential of this material. The GPMT was conducted according to OECD Guideline 406, and Good Laboratory Practices were followed. Preliminary testing determined dose levels for the main study. Exposure during the induction phase of the GPMT consisted of a pair of intradermal injections with 3% musk ketone followed by a 48-hour occlusive dermal application with 75% musk ketone seven days later. After a 2-week rest period, the challenge phase consisted of 24-hour occluded dermal applications with three concentrations: 75, 25 and 7.5% musk ketone. The vehicle used for all phases was 9:1 olive oil/acetone. Skin sites were examined at 24 and 48 hours following patch removal, erythematous reactions were quantified and scored. No irritation responses were observed, and the test was maximized. At 75% 5/20 test animals and 1/10 control animals responded; and at both 25 and 7.5% 3/20 test animals and 0/10 control animals responded. According to OECD guidelines, this material is not considered a sensitizer requiring a label, since there was less than a 30% response rate.

803 INHIBITION OF FLK-1/KDR, PDGFR AND FGFR DOES NOT ALTER HEALING OF INCISIONAL SUTURED SKIN WOUNDS IN MICE.

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The control of angiogenesis during tissue repair events is of crucial importance and disturbances of this process have been suggested to play a role in impaired skin wound healing. Vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) are the major angiogenesis factors, although other growth factors can also modulate angiogenesis during wound repair. Using an incisional wound model in SKH1 hairless mice, we evaluated the effects of therapeutic and supratherapeutic doses of SU006668, an inhibitor of PDGFR (Ki=0.008 µM), Flk-1/KDR (Ki=2.1 µM) and FGFR (Ki=1.2 µM), on the healing of skin wounds. Mice were administered vehicle, SU006668 (100 mg/kg/day and 400 mg/kg/day, b.i.d.), or dexamethasone (1 mg/kg/day; positive control) continuously for 6 days prior to wounding and for 28 days post wounding. A full-thickness incision was made on the dorsum of mice and closed with three equidistant sutures. Wound healing was monitored histologically and using a tensiometer up to 4 weeks post-wounding. SU006668 at therapeutic doses (100 mg/kg) had no significant effect on the tensile strength and macroscopic and microscopic morphology of the wounds, whereas dexamethasone treatment resulted in skin atrophy, decreased tensile strength, and delayed epidermal and dermal restoration. At supratherapeutic doses (400 mg/kg), where SU006668 may non-specifically inhibit other receptor tyrosine kinases, there were subtle transient histologic changes and slight decreases in tensile strength of minimal biological significance, suggesting a slight delay in the wound healing process up to 14 days post-wounding with complete return to control levels thereafter. In conclusion, these data indicate that PDGFR, Flk-1/KDR and FGFR inhibition at levels necessary to inhibit tumor growth in mouse xenograft models, does not affect the healing of incisional wounds and that redundant pathways likely compensate for inhibition of PDGF, VEGF and FGF function in normal cellular processes.

804 EFFICACY OF DIPHOTERINE HCL DECONTAMINATION IN RATS: A COMPARATIVE STUDY.

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Efficacy of Diphoterine(R) HCl Decontamination in Rats. A comparative study. Cavallini M, Corsi MM, Mathieu L, Blomet J, Hall AH. Twenty-five male rats had dermal exposure to 25% HCl for 15 seconds. Five control rats had no decontamination. All applicable animal use guidelines were followed. The other groups of 5 rats each were decontaminated as follows: saline solution; calcium gluconate; Diphoterine. Assays of IL-6, TNF-alpha, NO, and substance P indicated that Diphoterine was more efficacious than the other decontamination solutions.

805 CALIBRATION OF *IN VITRO* DERMAL ABSORPTION TEST SYSTEMS: INTER-SYSTEM VARIATION IN TRITIATED-WATER KP VALUES?

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The skin permeability coefficient (Kp) is used to predict dermal systemic exposure to environmental contaminants. Since many different *in vitro* dermal absorption test systems have been reported in the literature for measuring Kp values, our laboratory conducted inter-system tests for calibration purposes. Tritiated water (^3H_2O) was selected as the test chemical since the Kp of ^3H_2O is often used to test human skin barrier integrity. Our tests were conducted following standard OECD guidelines. Three different flow-through type *in vitro* test systems were used; the Bronaugh, Amie and AIDA systems. The Bronaugh and Amie systems were obtained commercially, the Automated *In-vitro* Dermal Absorption (AIDA) system was developed in-house. All three systems used flow-through donor and receivor solutions except for the Bronaugh system which used a non-flowing static donor. The Bronaugh, Amie and AIDA systems demonstrated average ^3H_2O Kp values (units of cm X 10^3/hr) of 2.3 ± 1.38 (n = 6), 1.0 ± 0.40 (n = 6), and 0.5 ± 0.11 (n = 4), respectively. Normalized for the Bronaugh Kp data, the average Kp was 2.4 and 4.5 fold lower for the Amie and AIDA systems, respectively. As tests were conducted with fresh/unfrozen human breast skin from the same donor and all experimental conditions (e.g. temperature, donor and receivor solution concentrations/compositions) were kept the same, the reason for an intra-system difference in Kp values was not apparent. The higher Kp obtained for the Bronaugh system was not considered to be due to its use of a static donor supply since a flowing donor should act to stir the donor solution, and if anything increase the absorption rate. Given the range in standard deviation in the average Kp data reported here, further corroboratory tests are required with ^3H_2O and other chemicals to examine the need for developing inter-system calibration factors.

806 DIETHANOLAMINE (DEA) *IN VITRO* DERMAL ABSORPTION IN FUZZY RAT SKIN.

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Diethanolamine (DEA) is used in cosmetic formulations as an emulsifier, thickener, wetting agent, detergent, and foam booster. DEA is reported to be used in mainly wash-off cosmetic formulations. DEA is also used in the preparation of

DEA conjugates (DEA condensates and DEA salts). DEA conjugates are used almost entirely in wash-off products, but there are uses reported in leave-on products. Unreacted DEA concentrations in DEA condensate cosmetic raw materials may be as high as 20%. Concerns about DEA's safety have been raised by National Toxicology Program carcinogenicity testing in rodents. Therefore, we initiated *in vitro* studies to measure DEA fuzzy rat dermal absorption. [^{14}C]DEA (approx. 0.1 μCi/cell) skin absorption was measured by using one dosing vehicle which was a spiked consumer lotion product applied at 3.0 mg/cm^2. The dosing vehicle containing DEA was applied to skin in the diffusion cells for 24 hr, then the skin was washed. Absorption was measured over a period of 72 hr by using flow-through diffusion cells (0.64 cm^2) with a receptor fluid consisting of HHBSS (pH 7.4). In rat skin(n=3), the percentage of applied dose absorbed over 24 hr was 1.4 ± 0.5 (mean ± SEM) with approximately 4% remaining in skin. At 72 hr after DEA application, the percentage of applied dose absorbed was 1.6 ± 0.5 with approximately 4% remaining in skin. At 24 hr, there was 1.9 ± 0.8 and 1.9 ± 0.5 of the applied dose remaining in the stratum corneum and viable epidermis/dermis, respectively. Skin levels of DEA did not change even when followed for 72 hr. These studies indicate that little of the DEA that was found in the skin at 24 hr diffused through the skin to be absorbed into the receptor fluid. Therefore, skin levels of DEA should not be included in the estimate of systemic absorption used in an exposure assessment.

807 *IN VITRO* PERCUTANEOUS ABSORPTION OF DIETHANOLAMINE (DEA) IN HUMAN SKIN.

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Diethanolamine (DEA) is an alkanolamine contained in pharmaceuticals and various cosmetic and personal care products either as a conjugate (DEA condensates and DEA salts) or as a constituent of triethanolamine (TEA). Concerns about the safety of DEA have been raised by the National Toxicology Program (NTP). Therefore, we initiated studies to measure the extent of DEA absorption in human skin relevant to exposures from consumer products. DEA penetration was determined from three different product classes: shampoos, hair dyes and body lotions. Two commercial products from each class were spiked with [14C]DEA and applied to excised viable and non-viable human skin mounted in flow-through diffusion cells maintained with a physiological buffer (HHBSS, pH 7.4) as receptor fluid. Products remained on the skin for 5 min, 30 min and 24 hours for shampoos, hair dyes and body lotions, respectively. At the end of 24 hours, the amount of DEA absorbed in skin layers and the receptor fluid was determined and expressed as the percent of the applied dose absorbed for each product type. Most of the absorbed dose was found in skin: 2.8%, 2.9% and 10.0% for shampoos, hair dyes and body lotions respectively. Only small amounts penetrated into the receptor fluid: 0.08%, 0.09% and 0.9% for shampoos, hair dyes and body lotions respectively. In skin absorption studies extended to 72 hours only small amounts of additional DEA were absorbed into the receptor fluid. Therefore, skin levels of DEA should not be included in estimates of systemic absorption used in exposure assessments. Substantially more systemic absorption of DEA would be expected when applied in a leave-on product. In daily repeat dose studies with a lotion, DEA penetration into receptor fluid increased slightly but not significantly from 0.47% (day 1) to 0.76% and 0.88%, on the second and third days respectively. A [3H]water test indicated that the barrier of the skin remained intact throughout the study. DEA appeared to accumulate in the skin (29.2%) with very little diffusing out into the receptor fluid.

808 RISK ASSESSMENT OF ORGANOPHOSPHORUS CHEMICALS INCLUDING CHEMICAL WARFARE AGENTS.

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The objective was to perform analysis on existent dermal absorption data on selected organophosphorus compounds including chemical warfare agents – sarin (GB), soman (GD) and VX – and develop a postulate of critical factors in observed regional differences in toxicant uptake/absorption by human skin. Body contours for skin absorption and lethality were developed. Parathion and malathion exposed head and neck region (x4), trunk (x3) and genital area (x12) absorb more chemical than arms and hands, and legs and feet. In field use, parathion has caused human death while malathion is considered safe. Permeability constants (Kp) (potential chemical absorbed through human skin per unit area and time) indexed to regional variation gives the mass of chemical absorbed through all regions of the human body. Human skin is more permeable to parathion than malathion. Further overlap of toxicity data to the construct showed lethality with parathion but not for malathion. VX has a high Kp and is also the most toxic; 50% lethality is reached not only for total systemic exposure but also for just trunk exposure in the first hour. In later hours, 50% lethality is reached when exposure is to any single region of the body. Sarin and soman are less toxic percutaneously than VX, and they have the same toxicity level. However, soman has greater skin absorption than sarin. Estimates of 50% lethality are only reached for sarin at the 24-hour exposure level, whereas the 50% lethality estimate for soman is reached in the first hour. Chemical warfare agents are a serious threat to human life. In order to minimize or prevent the hazard of these agents, we need to understand the way that these chemicals get onto and into the human body, both for military function and protection of life.

809 EVALUATION OF AN *IN VIVO* MODEL: HUMAN SKIN GRAFTED ON "NUDE" MICE TO PREDICT DERMAL ABSORPTION IN HUMAN.

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Dermal absorption data from an *in vivo* model based on human skin grafted on "nude" mice were compared with data from human volunteer studies and two other models currently used (rat *in vivo* model and *in vitro* human skin model using diffusion cell) for three lipophilic chemicals. Results indicate that for lindane and cypermethrin dermal penetration and diffusion were similar for the human skin grafted on "nude" mice and diffusion cell models. For malathion, although similar diffusion was found in *in vitro* and grafted human skin, the amount remaining in *stratum corneum* (SC) was three times higher with diffusion cell model. Rat skin is more permeable than human skin independently of the human skin model used. Absorption through human skin in diffusion cell and human skin grafted on mice were two times higher than absorption in human volunteers. Inclusion of SC content in total absorbed dose would markedly overestimate absorption in human volunteers for malathion and cypermethrin. So, the fate of the high amount of cypermethrin in SC has been evaluated both with human skin grafted on mice and the rat *in vivo*. Four days after decontamination, 15.4% of cypermethrin was still present in SC of rat skin compared to only 2.5% in human skin grafted on "nude" mice. Further, results with human skin grafted on "nude" mice indicate that only one-third of the quantity of cypermethrin remaining in SC at the end of exposure period was absorbed while the remainder was presumably lost through desquamation. This work indicates that the diffusion cell using human skin is a good model for assessing absorption of chemicals. However for a lipophilic compound stored in *stratum corneum*, an *in vivo* model like the human skin grafted on "nude" mice may predict more accurately the level of absorption in human.

810 ALLERGEN-INDUCED CHANGES IN INTRACELLULAR EPIDERMAL TUMOR NECROSIS FACTOR α EXPRESSION.

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Following topical exposure of mice to skin sensitizing chemicals, mRNA expression for a number of epidermal cytokines is upregulated, including that for tumor necrosis factor α (TNF-α). To measure directly intracellular TNF-α protein at the single cell level, a two-colour flow cytometric method for the detection of cytokine-producing keratinocytes and Langerhans cells (LC) prepared *ex vivo* from BALB/c strain mice has been developed. We report that the process of trypsinization of dorsal ear halves to prepare epidermal cell suspensions results in the production of TNF-α by the majority of keratinocytes, with the subsequent induction of other epidermal cytokines. However, the inclusion of monensin during epidermal cell preparation and fixation early in the isolation procedure with formaldehyde reduced significantly non-specific upregulation of TNF-α expression. Using this approach, permeabilization of epidermal cell suspensions with saponin and two-colour staining for a number of cytokines together with MHC class II revealed approximately 3-5% of keratinocytes derived from naive mouse skin to be positive for TNF-α. Under these conditions it was also found that a proportion of keratinocytes (approximately 20%) was associated with interleukin (IL)-6 and IL-12 expression, with approximately 40% exhibiting IL-10. At least 70% of LC were positive for intracellular IL-6 and IL-10, with only a minority population of LC displaying intracellular IL-12. Following topical application on the dorsum of both ears with the contact allergen oxazolone (Ox; 0.5%), enhanced intracellular TNF-α expression by a small proportion of keratinocytes, relative to vehicle treated mice, was observed within 30 minutes of exposure. Further increases of up to 10% in the frequency of keratinocytes positive for TNF-α were detected 1-4 hours following exposure to allergen. Taken together these data suggest that this technique provides a useful approach to the investigation of changes induced in epidermal cytokine expression as a result of skin sensitization.

811 EVALUATION OF THE DERMAL ABSORPTION OF METHYL ETHYL KETONE IN F344 RATS USING REAL-TIME BREATH ANALYSIS AND PBPK MODELING.

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Methyl ethyl ketone (MEK) is an industrial solvent found as a component of a variety of paints and coatings. Dermal exposure to MEK can result from the occupational or household use of paints and other commercial products. To understand the significance of these exposures, the dermal bioavailability of MEK was assessed in F344 male rats using a combination of real-time exhaled breath analysis and physiologically based pharmacokinetic (PBPK) modeling. Animals were exposed to MEK at a 5 mg/ml aqueous concentration using a 2.5-cm diameter occluded glass patch system attached to a clipper-shaved area on the back of the rat. Immediately following exposure the animal was placed in a glass off-gassing chamber and exhaled breath was monitored as chamber concentration in real time using an ion trap mass spectrometer (MS/MS). This real-time methodology was able to discern the uptake, peak concentration and clearance phases associated with the dermal exposure. For example, the exhaled breath profile clearly demonstrated the rapid absorption of MEK, with peak chamber concentrations ranging from 20 to 25 ppm observed within 30-60 minutes from the start of exposure. The PBPK model describing the exposure and off-gassing system was used to estimate a single dermal permeability coefficient to describe all the sets of exhaled breath data from n=6 animals. These rat studies using aqueous MEK will form the basis for comparing the dermal bioavailability of MEK in various paint products and may ultimately aid in understanding human health risk under a variety of exposure scenarios. (Supported by NIOSH 1-RO1-OH03658-01A2).

812 ASSESSMENT OF DERMAL IRRITATION OF THREE BENZENE SULFONATE COMPOUNDS.

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Three benzene sulfonate compounds are present in groundwater near a former disposal site at concentrations ranging from <1 mg/L to <500 mg/L. Dermal irritation studies were performed for each of the three sulfonate compounds to determine if they would cause irritation to the skin of persons using water containing the compounds for bathing, showering, or other uses where skin would be exposed. The three compounds are: (1) benzene sulfonate; (2) benzene meta-disulfonate; and (3) para-hydroxybenzene sulfonate. The studies were performed in accordance with USEPA's Health Effects Test Guidelines: OPPTS 870.2500, Acute Dermal Irritation, 1998. At the highest dose tested (5, 000 mg/L), all three sulfonate compounds were considered to be slight irritants, producing very slight to mild erythema. In all cases, the reactions were reversible. At the second highest dose tested (2, 000 mg/L), benzene meta-disulfonate and para-hydroxybenzene sulfonate caused no irritation and were considered not to be irritants. At the second highest dose only benzene sulfonate is considered a slight irritant, producing a mild erythema that was completely reversible within 24 hours. Benzene sulfonate is not considered an irritant at 1, 000 mg/L or at 500 mg/L. It is important to note that all three sulfonate compounds produced only a slight irritation at the highest dose tested. No compound produced edema or a severe irritation (i.e., severe erythema). Furthermore, all irritation responses at the highest dose tested were reversible within 72 hours, and the only irritation response observed at the second highest dose (2000 mg/l of benzene sulfonate) was reversible in less than 24 hours.

813 ASSESSMENT OF DERMAL PENETRATION OF THREE BENZENE SULFONATE COMPOUNDS.

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Three benzene sulfonate compounds are present in groundwater near a former disposal site at concentrations ranging from <1 mg/L to <500 mg/L. Dermal penetration studies were performed for each of the three sulfonate compounds to determine if they would be systemically absorbed by persons using water containing the compounds for bathing, showering, or other uses where skin would be exposed. The three compounds are: (1) benzene sulfonate (BSA); (2) benzene meta-disulfonate (BDSA); and (3) para-hydroxybenzene sulfonate (HBSA). The dermal penetration studies with infinite doses of 2, 000 mg/L aqueous solutions were performed *in vitro* for 24 hours with human abdominal skin using Franz diffusion cells with 0.9% saline solution as receptor fluid. Each compound was tested with six skin samples from a minimum of three donors. The integrity of human skin was tested with 3H-water before use. Aliquots of receptor fluid were analyzed for the presence of the test compounds using ion-pairing reverse phase high performance liquid chromatography (HPLC) and ultraviolet absorption. HPLC conditions were 53:47, 25 mM tetrabutylammonium bromide:methanol (isocratic). Analytical wavelengths were 263, 267 and 271 nanometers for BSA, BDSA and HBSA, respectively. No sulfonate compounds were detected in the receptor fluid at 24 hours in any of the test cells. The Estimated Quantitation Limit (EQL) for all compounds was determined to be 0.5 ng/ul. Assuming that the compounds were present at one-half the EQL, the permeability constants, Kp, were 2E-05 cm/hour. The results of this study confirm the results of a pilot study performed using the same methods but different chromatographic conditions.

814 MECHANISMS OF UVB LIGHT-INDUCED SUPPRESSION OF NITRIC OXIDE PRODUCTION IN MURINE KERATINOCYTES.

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Ultraviolet light of high energy and shorter wavelengths (UVB, 290-320 nm) is utilized therapeutically to reduce irritation in inflammatory dermatosis. The mechanisms underlying the actions of UVB are unknown. We hypothesize that the UVB light suppresses oxidant generation in the skin. Nitric oxide is a highly reactive oxidant generated by keratinocytes by an inducible form of nitric oxide synthase (NOS2) in response to the inflammatory cytokine, gamma interferon. In the present studies we examined the effects of UVB light on NOS2 activity in keratinocytes. We found that UVB light suppresses gamma-interferon-induced nitric oxide production in these cells. This effect was dose-dependent in the range of 2.5-25 mJ/cm^2. This was found to be due to inhibition of NOS2 mRNA and protein expression in the cells, as determined by RT-PCR and western blotting, respectively. Two transcription factors known to be important in regulating expression of NOS2 are signal transducer and activator of transcription-1 (STAT-1) and nuclear factor κB (NF-κB). Using electrophoretic mobility shift assays, we found that gamma interferon readily induced STAT-1 activity while NF-κB was constitutively expressed in the cells. UVB light (25 mJ/cm^2) was found to cause a 30-40% reduction in the activation of these transcription factors. Nuclear translocation of the p50 and p65 subunits of NF-κB was also marked reduced by UVB light. Taken together, our data suggest that UVB light functions to suppress expression of NOS2 by inhibiting activation of key transcription factors important in regulating expression of the NOS2 gene. Decreased expression of NOS2 and subsequent nitric oxide production in keratinocytes may be an important mechanism for the anti-inflammatory and therapeutic actions of UVB light. Supported by NIH grant ES 06897.

815 THE EFFECTS OF CHRONIC ALCOHOL CONSUMPTION ON DERMAL PENETRATION OF PESTICIDES.

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The skin is the major source of xenobiotic exposure in occupational settings. Topically applied ethanol is commonly used as a dermal penetration enhancer. The hypothesis of this work is that ethanol, consumed orally, will also behave as a dermal penetration enhancer. A series of four pesticides, paraquat (MW = 257, log K_{ow} = -4.5), 2, 4 D (MW = 221, log K_{ow} = 2.8), atrazine (MW = 216, log K_{ow} = 2.3) and trifluralin (MW = 335, log K_{ow} = 5.1) were selected as model compounds to test this hypothesis. These compounds were chosen for their diverse octanol water partition coefficients (K_{ow}), a factor known to influence percutaneous penetration. Male Wistar rats were fed a diet of 36% ETOH (25 mM in blood) or control in a liquid diet for 4-8 weeks. The animals were then sacrificed, their skin shaved, removed and placed in an *in vitro* Bronaugh Style flow through diffusion chamber. The test compound was spiked with ^{14}C labeled pesticide and placed on the epidermis for 24 hours. Buffer flowing past the dermal side of the skin was collected in 90-minute fractions and counted *via* liquid scintillation. Chronic ethanol consumption significantly enhanced dermal penetration for the three most hydrophilic compounds tested ($p<0.05$). The penetration of trifluralin, the most hydrophobic pesticide, was inhibited by chronic ethanol consumption ($p<0.05$). A regression analysis demonstrates a correlation between hydropholicity and enhancement of transdermal penetration by chronic ethanol consumption. Chronic ethanol consumption, therefore, leads to compromised dermal barrier in rats resulting in increased penetration of pesticides. These studies imply that alcoholic workers have greater dermal exposure of chemicals than previously estimated.

816 TRANSCRIPT PROFILING OF MURINE LYMPH NODE CELLS : ALLERGEN-INDUCED EARLY GENE CHANGES.

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In order to identify early events induced in lymphoid tissue following topical treatment of mice with contact allergen, gene expression changes in draining lymph node cells (LNC) have been profiled using DNA microarrays. BALB/c strain mice

were exposed topically to the potent skin sensitizer 2, 4-dinitrofluorobenzene (DNFB; 0.5%) on the dorsum of both ears. Control mice were exposed to vehicle (4:1 acetone:olive oil) alone. At 18 or 48 hours post-exposure, draining LNC were isolated and the mRNA prepared. Radiolabelled cDNA probes were generated from mRNA samples and hybridized to in-house nylon microarray filters comprising of 8734 murine gene sequences arrayed in duplicate (3336 assigned to known genes, 5398 expressed sequence tags [ESTs]). Changes in gene expression were calculated as the ratio of values for LNC isolated from DNFB-treated animals compared with LNC from concurrent controls (changes of 1.5 fold or greater were considered significant). At 18 hours post-exposure, the expression of more than 95% of represented genes was unchanged by DNFB treatment. However, a number of consistently down-regulated genes was identified, including several ESTs and glycosylation-dependent cell adhesion molecule 1 (GlyCAM-1). Further analysis of tissue sampled 48 hours after exposure to DNFB provided examples of both up- and down-regulated genes including onzin and guanylate binding protein-2, and GlyCAM-1 respectively. Induced changes in these three genes were confirmed over a more extensive time course (18 to 120hr) using Northern blotting and/or RT-PCR analyses, indicating that changes in gene expression of GlyCAM-1, onzin and possibly GBP-2 are robust markers of early changes in the lymph node provoked by contact allergen. In subsequent experiments the selectivity of these markers for contact allergens will be investigated.

817 SUBCHRONIC TOXICITY OF 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) OBEYS HABER'S C X T RULE AFTER ORAL ADMINISTRATION.

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Groups of 8 to 11 A/J mice were administered a loading dose of 100, 150 or 200 microg/kg of TCDD followed by weekly maintenance doses of 15.0, 22.5, or 30 microg/kg by gavage in corn oil. A positive control group (n=10) received a single dose of urethane (1 mg/g). All positive control mice developed by 4 months after dosing adenocarcinomas of the lung. None of the TCDD-treated animals developed adenocarcinomas of the lung by 6 months after initial dosing. On day 76 the first mouse died in the highest dose group and on day 226 the last mouse died in the lowest dose group of nonspecific subchronic toxicity. Mean time to deaths were 178 ± 7.9 (S.E.), 137 ± 7.6 and 116 ± 7.3 days for the 100, 150 and 200 microg/kg initial dose groups, respectively. The c x t products for the respective dose groups were 83.3 ± 7.1, 78.1 ± 6.9 and 77.7 ± 8.2 microg•day. The hypothesis that TCDD will cause adenocarcinomas in A/J mice without initiation has been refuted. However, under conditions of kinetic steady state once again the fundamental law of toxicity emerged with a mere 1.8% variability in still another species. This experiment provides further support for the notion that Haber's rule is not limited to inhalation but is generalizable for all studies conducted under conditions of kinetic steady state.

818 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) EXPOSURE OF MALE MOUSE UROGENITAL SINUS IN EXPLANT CULTURE IMPAIRS PROSTATIC BUD FORMATION.

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in utero TCDD exposure on Gestation Day (GD) 13 impairs prostatic bud formation from the urogenital sinus (UGS) of the C57Bl/6J mouse fetus on GD 18. Our objectives were to determine: (1) if the inhibitory effect of TCDD on prostatic bud formation following *in vivo* exposure also could be produced by *in vitro* exposure of GD 14 UGS explants in organ culture for 5 days to TCDD, and (2) whether the inhibition of budding, if observed, was AhR-dependent. Prostatic bud formation from the fetal mouse UGS is androgen-dependent. On GD 14 the UGS from wild-type and AhR knock-out (AhRKO) C57Bl/6J male fetuses were removed and cultured in 5% charcoal-dextran stripped FBS supplemented with either vehicle (Control), 10^{-8} M testosterone (T), or 10^{-8} M testosterone and 10^{-9} M TCDD (T+TCDD). Media were changed every 48 hr. UGS explants were harvested after 5 days in culture and examined for prostatic bud formation by scanning electron microscopy. In Control UGS explants derived from both wild type and AhRKO fetuses only 2 dorsolateral buds and no ventral buds were observed. This lack of prostatic bud formation was expected and is consistent with previous studies showing that this is an androgen-dependent process. Thus, in the absence of androgen stimulation virtually no buds were observed in either of the two Control groups (wild type and AhRKO). Supplementation of the culture medium with T caused formation of DLBs and VBs in explants of both genotypes, wild type and AhRKO. In UGS explants exposed to T+TCDD the effect on budding was genotype-dependent. In wild type UGS explants exposed to T+TCDD prostatic bud formation resembled that of Control wild type UGS explants that received no androgen stimulation. On the other hand, UGS explants obtained from AhRKO fetuses and exposed to T+TCDD were similar to UGS explants obtained from AhRKO fetuses exposed to T only. Collectively these results demonstrate that TCDD can act directly on the UGS to disrupt prostatic bud formation by a mechanism that is AhR-dependent. (NIH grant ES01332)

819 ALTERED SENSITIVITY OF THE MOUSE FETUS TO IMPAIRED PROSTATIC BUD FORMATION BY DIOXIN: INFLUENCE OF GENETIC BACKGROUND AND NULL EXPRESSION OF TGFα AND EGF.

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2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) inhibits prostatic bud formation from the urogenital sinus in the C57Bl/6J mouse fetus. Knock-out EGF (EGFKO), TGFα (TGFαKO), and EGF+TGFα (EGFKO/TGFαKO) mice were used to investigate the roles of EGF and TGFα in the impairment of prostatic budding by TCDD. The EGFKO and EGFKO/TGFαKO mice share a "mixed" genetic background of the 129 and C57Bl/6J strains; whereas genetic background of TGFαKO mice resembles the C57Bl/6J strain. Compared to the wild-type C57Bl/6J strain (WT), wild-type mice of the "mixed" genetic background (WT-m) required about 100 times more TCDD to produce an equivalent inhibitory effect on prostatic budding following *in utero* TCDD exposure. On gestation day (GD) 12, timed pregnant WT and TGFαKO dams were dosed with 0, 0.2, and 1 μg TCDD/kg and dams producing WT-m, EGFKO, and EGFKO/TGFαKO offspring were dosed with 0, 1, 10, 50, 100, or 150 μg TCDD/kg. Essentially complete agenesis of ventral buds (VBs) and a reduction in dorsolateral bud (DLB) numbers were observed in 1 μg/kg TCDD-exposed WT and TGFαKO fetuses on GD 17.5, suggesting that the absence of TGFα does not affect responsiveness to TCDD. WT-m fetuses showed no inhibition of budding at 1 μg TCDD/kg, but at 50 μg/kg and higher doses of TCDD there were dose-related reductions in VB and DLB number. The 10 μg TCDD/kg EGFKO group showed an inhibition of VB and DLB formation similar to the 150 μg TCDD/kg WT-m group. EGFKO and EGFKO/TGFαKO groups treated with 150 μg TCDD/kg showed agenesis of VBs and nearly complete agenesis of DLBs. Sensitivity to TCDD-induced impairment of prostatic budding is enhanced in EGFKO and EGFKO/TGFαKO fetuses. Thus, null expression of EGF appears to increase sensitivity of TCDD-inhibited prostatic bud formation. (Supported by USEPA and NIH grant ES01332)

820 PROTECTION BY VITAMIN E SUCCINATE OF TCDD-INDUCED OXIDATIVE STRESS IN RATS.

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TCDD is known to induce oxidative stress in hepatic, as well as brain tissues of rats after acute, subchronic and chronic exposures. In this study we demonstrate that subacute treatment of rats with TCDD can also induce oxidative stress in the same tissues, and that vitamin E succinate can provide protection against this effect. Animals were randomly assigned to four different groups, including a control (C), TCDD-treated (T-treated), vitamin E succinate-treated (E-treated) and TCDD plus vitamin E-treated (T + E-treated) group. All treatments were given by gavage, daily for 30 days. The T- treated group received 100 ng TCDD/kg/day, while the E-treated group received Vitamin E-succinate at doses of 150 mg/kg/day for the first three days, followed by doses of 40 mg/kg/day for 27 days. However, The T + E-treated group received combined dose regimens of the forementioned T- and E-treated groups, and control group received corn oil vehicle used to dissolve TCDD and vitamin E succinate . Animals were sacrificed at the end of the treatment period and brain and hepatic tissues were assayed for various biomarkers of oxidative stress including production of superoxide anion (SA), lipid peroxidation (LP) and DNA damage. While subacute treatment with TCDD produced significant production of SA and LP, with significantly more effects produced in hepatic as compared with brain tissues, it didn't produce significant DNA damage in either tissues. The results also indicate that vitamin E-succinate provided significant protection against TCDD-induced SA and LP production in hepatic and brain tissues, with significantly more protection provided in brain as compared with hepatic tissues.

821 LOW DOSE PRENATAL DIOXIN EXPOSURE IN RATS INDUCES SEXUALLY DIMORPHIC CHANGES IN OPERANT BEHAVIOR AND BRAIN MORPHOLOGY.

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Prenatal exposure to high doses of dioxin(TCDD;2, 3, 7, 8-tetrachloro- dibenzo-*p*-dioxin) induces changes in motor activity and cognitive and sexual behavior. In an earlier study, prenatal exposure in rats to a single low dose of TCDD on gestational

day 18 (GD18) produced a significant dose-related reduction in wheel running operant behavior. In addition, a quantitative study of brain morphology showed that TCDD also altered normal patterns of sexually dimorphic asymmetries in cortical thickness. The current study examined exposure to low, environmentally relevant levels of TCDD at an earlier stage of gestation. It employed two endpoints: schedule-controlled operant behavior based on lever-pressing for food reinforcement, and cell counts in selected areas of cerebral cortex. Pregnant Spraque-Dawley rats were given a single p.o. dose of 0, 20, 60 or 180 ng TCDD/kg on GD 8. Male-female pairs of offspring 90 days old from all dose groups were trained on two operant procedures: (1) an incremental fixed ratio (FR) schedule, and (2) a multiple FR/differential reinforcement of low rate schedule (mult FR/DRL). TCDD evoked sexually dimorphic response patterns in both procedures. Exposed males responded at lower rates than male controls, while exposed females responded at higher rates than their controls. Cortical cell counts from areas 17, 18a and 39 in bregma -3.9 from offspring exposed to 180 ng/kg, the only dose examined so far, exhibited significant reversal of hemispheric dominance in both males and females. These data demonstrate that prenatal exposure to low environmental doses of TCDD evokes sexually dimorphic patterns of operant behavior and cortical asymmetry in a manner consistent with previous reports. (Funded by ES08958 and ES01247 from NIEHS.)

822 EFFECTS OF TCDD, 2, 3, 4, 7, 8-PCDF AND PCB 126 ON ESTROGEN HYDROXYLATION IN MALIGNANT (MCF-7) AND BENIGN (MCF-10A) HUMAN MAMMARY TUMOR CELLS.

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Mounting evidence suggests a role for estrogen metabolites in the etiology of breast cancer. In human breast tissue, estrogens are mainly hydroxylated by cytochrome P450 1A1 (CYP1A1, estrogen 2-hydroxylase) and P450 1B1 (CYP1B1, estrogen 4-hydroxylase). In several studies, the 4-hydroxylated but not the 2-hydroxylated metabolites of estrogens were found to be carcinogenic. This study investigated the effects of several dioxin-like compounds on estrogen hydroxylase activity in human mammary tumor cell lines MCF-7 (adenocarcinoma) and MCF-10A (fibrocystic disease). Cell cultures were exposed to various concentrations of 2, 3, 7, 8-TCDD, 2, 3, 4, 7, 8-PCDF or PCB 126. After 72 hours the media were replaced with media containing 1 µM estradiol. Estrogen 2- and 4-hydroxylase activities were determined by measuring 2- and 4-methoxylated estrogen levels by GC/MS. Both the constitutive and induced estrogen hydroxylase activities were lower in MCF-10A than in MCF-7 cultures. This was also true for ethoxyresorufin O-deethylase (EROD) activity in both cell lines. In MCF-7 cells, estrogen metabolism was induced in a dose-dependent manner with relative potencies in the order TCDD ≡ PCDF > PCB 126, conform the WHO TEF values. Induction of estrogen metabolism was mainly attributed to induction of 2-hydroxylation of estradiol. In MCF-10A cells, only TCDD induced estrogen metabolism markedly, while PCDF and PCB 126 appeared to have only a moderate inductive effect. Induction of estrogen metabolism by TCDD mainly resulted in elevated levels of both 4- and 2-hydroxylated metabolites of estrone. These data suggest a difference in response to dioxin-like compounds between malignant and benign mammary tumor cell lines. Studies on the effects of several other dioxin-like compounds on estrogen metabolism and mRNA levels of CYP1A1 and CYP1B1 in MCF-7 and MCF-10A cells are in progress.

823 TCDD-INDUCED ABNORMAL DEVELOPMENT OF PAWS IN TCDD-RESPONSIVE LACZ TRANSGENIC MICE.

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The aryl-hydrocarbon Receptor (AhR) is a ligand-dependent transcription factor that mediates the toxicity of halogenated aromatic hydrocarbons including 2, 3, 7, 8-tetra-chlorodibenzo-*p*-dioxin (TCDD). These compounds are potent reproductive and developmental toxicants due to their ability to modulate gene expression and cellular differentiation/proliferation. Determination of tissue-specific AhR transcriptional activity *in vivo* will allow the identification of tissues/cells where the AhR may have some functional role susceptible to disruption by TCDD exposure. We have developed a transgenic mouse model containing an AhR-responsive reporter that can delineate the temporal and spatial context of active AhR in a cell-/region-specific manner. This model has identified several targets tissues during gestational days (GD) 13 to 16 including the lung, liver, genital tubercle, and paws after 24h TCDD exposure. Most transgene induction is in tissue that have been previously identified as target tissues, with the exception of the paw, a novel target. We hypothesize that TCDD-induced AhR activity in developing paws of mice is associated with alterations in gene expression and aberrant development. Pregnant females were exposed to TCDD (30 µg/kg) on GD 14. Twenty-four hours later and on post-natal day (PND) 2 pups were evaluated at a morphological and gene expression level. Significant differences were identified upon morphometric analysis of the paw at PND 2 in both epithelial cell layer and digit thickness, with TCDD exposed tissues being increased in both cases. Preliminary microarray analysis identified novel genes as well as known dioxin-responsive genes (e.g. CYP1B1) in the paws of day 15 embryos that were affected by TCDD exposure. The results indicate that the model is capable of detecting AhR activation, thus allowing the identification of developmental cell and gene targets of TCDD. (Funded by NIH grant ES0943, Training grant 07026, and Center grant 01247).

824 EFFECT OF TCDD ON THE SURVIVAL OF BONE MARROW-DERIVED DENDRITIC CELLS *IN VITRO.*

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Previous studies in our laboratory have shown that an immunosuppressive dose of TCDD, in the absence of antigen, activates splenic dendritic cells (DC) *in vivo*. However, recovery of DC was subsequently reduced approximately 40%. We hypothesized that TCDD sensitizes DC to Fas-mediated apoptosis. To demonstrate a TCDD effect on DC survival, we used an *in vitro* bone marrow-derived dendritic cell (BMDC) model. On day 0, cells from the bone marrow of femurs from C57Bl/6 mice were cultured in media containing 20% FBS and GM-CSF for 6 days. TNF-α was added on day 6 to generate a mature DC phenotype. On day 7, media and cytokines were removed and replaced with media containing 5% FBS and vehicle or TCDD. On day 8, agonistic anti-Fas (Jo-2) or control IgG antibodies were added to the cultures. On day 9, the cells were assayed for apoptosis using Annexin V and 7-AAD and analyzed using flow cytometry. In separate experiments, TCDD alone increased apoptosis or enhanced the degree of apoptosis induced by Fas ligation. In all experiments, the highest degree of apoptosis was seen when TCDD (10^{-8} M) and anti-Fas were combined. In order to determine if the increase in apoptosis was due to increased levels of Fas on the cell surface, we looked at the level of Fas expression on BMDC by flow cytometry. We found no difference in Fas expression between vehicle- and TCDD-treated cells, suggesting that TCDD may alter intracellular signaling pathways leading to apoptosis. Further studies are necessary to determine if the relationship between TCDD and Fas signaling is additive or synergistic. Supported by NIH Grants P01 ES00040 and P01 ES07060.

825 TIME-DEPENDENCE OF TCDD- AND HXCDD-INDUCED CYP 1A1 EXPRESSION AS MEASURED BY EROD ACTIVITY, WESTERN AND NORTHERN BLOTS.

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Female Sprague-Dawley rats (~250 g - Harlan) were housed in large polycarbonate cages with hardwood bedding. Feed and water were provided ad libitum to all animals. Rats were given a loading dose of either 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) or 1, 2, 3, 4, 7, 8-hexachlorodibenzo-*p*-dioxin (HxCDD) in corn oil *via* oral gavage (five animals per treatment group); controls were dosed with vehicle alone. In addition, animals were administered maintenance doses of TCDD or HxCDD every third or ninth day, respectively, to maintain pharmacokinetic steady state. Three iso-effective doses of TCDD/HxCDD (0.0125/0.3125, 0.05/1.25, or 0.2/5 microg/kg) were selected based on their toxic equivalence. Rats were terminated 2, 8, 32, or 64 days after receiving the loading dose. Livers were removed, weighed and snap frozen in liquid nitrogen. TCDD caused a dose- and time-dependent induction of EROD activity. There was about a 100-fold increase in EROD activity at constant time (day 2) and about an additional 5-fold increase at constant dose (day 64). The same samples that were used to measure EROD activity were also analyzed for CYP 1A1 mRNA and protein by Northern and Western blotting, respectively. For Western blots, a rabbit anti-rat CYP1A1 polyclonal antibody from XenoTech (Kansas City, KS) was used. Murine CYP1A1 cDNA was obtained from ATCC for use in Northern blots. Semi-quantitation of Western and Northern blot results were obtained using Gel Pro image analysis. Both CYP 1A1 mRNA and protein increased in a time-dependent manner in both the TCDD- and HxCDD-treated rat livers, in agreement with the induction of EROD activity. The CYP1A1 protein induction was approximately 2-fold between 32 and 64 days at constant dose; there was a greater (approximately 8 fold) increase in EROD activity in the same samples.

826 DIOXIN IMPAIRS OSMOREGULATION IN THE ZEBRAFISH LARVA.

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The most prominent sign of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) developmental toxicity in the zebrafish larva is edema. However, the cause of the edema and its relation to the other signs of TCDD toxicity in zebrafish is unknown. One

possibility is that the edema results from impaired osmoregulation, which allows water to move into the larva. To test this hypothesis, the osmolality of the water in which TCDD-exposed embryos underwent development was increased with the addition of 250 mM mannitol. Accordingly, embryos were exposed for 1 hr to vehicle or 10 ng TCDD/ml (LC100 dose) within 4 hours post fertilization (hpf). Embryos from the vehicle and TCDD groups, respectively, were then maintained throughout development in water either with or without osmotic support. Larvae from the 4 groups were examined for signs of toxicity at later stages of development. Increasing external osmolality had no significant effect on the incidence or severity of pericardial edema at 100 hpf, suggesting that this early stage of pericardial edema is not secondary to impaired osmoregulation. In contrast, osmotic support significantly reduced the incidence of pericardial edema in TCDD-exposed embryos at 130 hpf when compared to larvae without osmotic support and this protective effect was maintained through 150 hpf. Increasing external osmolality had an even greater protective effect against yolk sac edema than pericardial edema at 130 and 150 hpf when compared to that of larvae exposed to TCDD without osmotic support. In addition, there was a striking reduction in the severity of yolk sac edema and a less dramatic decrease in the severity of late stage pericardial edema in TCDD-exposed larvae with osmotic support. Osmotic support had no effect on either the incidence or severity of other endpoints of TCDD toxicity (reduced lower jaw length and decreased blood flow). Taken together, these results demonstrate that TCDD-induced yolk sac edema and late stage pericardial edema in the zebrafish larva are associated with impaired osmoregulation. (UW Sea Grant)

827 EFFECTS OF *IN UTERO* / LACTATIONAL EXPOSURE TO TCDD ON BONE IN RATS.

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We have previously shown that TCDD treatment alters bone geometry and decreases mechanical strength in adult rats. In this study bone geometry, mineral density and mechanical strength were examined in rats exposed to TCDD *in utero* / lactationally. Pregnant female rats of TCDD sensitive line C were given a single oral dose of TCDD (0, 0.03, 0.1, 0.3 or 1 μg/kg) on gestational day 15, and female offspring examined on postnatal day 35. Tibial and femoral diaphysis was scanned using a peripheral quantitative computed tomography (pQCT) system. Mechanical properties of tibial and femoral shaft and femoral neck was assessed using three-point bending test and axial loading, respectively. Body weights of offspring were slightly decreased at the highest dose-level only. Tibial and femoral length, as well as diaphyseal cortical and medullary cross-sectional areas were significantly decreased. In addition, cortical mineral density and polar moment of inertia were decreased. Some of these changes were dose-dependent, but reached statistical significance only at 1 μg/kg TCDD. Biomechanical testing revealed significantly decreased bending breaking force and bending stiffness of tibial and femoral shaft, and femoral neck. These changes were only seen at the highest dose level. The results indicate that bone is a sensitive target of dioxin toxicity, and that pre- and neonatal rats are clearly more sensitive to the bone effects than adult rats. (Supported by the Academy of Finland, the Finnish Research Programme on Environmental Health, Project 42551, and the European Commission, Contract QLK4-CT-1999-01446)

828 ADULT TCDD EXPOSURE AND EFFECTS ON MALE REPRODUCTIVE ORGANS IN THREE DIFFERENTIALLY TCDD SENSITIVE RAT LINES.

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2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) exposure during adulthood is known to cause adverse reproductive effects. The exceptional resistance of Han/Wistar (Kuopio; H/W) rats to acute lethality of TCDD is dependent on two different genes: the H/W-type mutated Ahr^{hw} and the other unknown B^{hw}. The resistance genes are separately expressed in three rat lines; line A (Ahr^{hw} alleles), line B (B^{hw} alleles) and line C (no resistance alleles). Some AHR-mediated effects (e.g. EROD) are insensitive (type 1 effects) to the alterations in resistance genes, while some (e.g. lethality) are sensitive (type 2 effects). To better understand the role of the resistance genes in TCDD toxicity, the significance of these genes in TCDD-caused reproductive defects were analyzed. Adult male rats were given a single oral dose of TCDD at 0-1000 μg/kg (line A), 0-300 μg/kg (line B), and 0-30 μg/kg (line C). Rats were killed and testes, epididymis, ventral prostate (VP) and seminal vesicles (SV) were collected and weighed. Cauda epididymis was weighed separately and frozen for sperm counts. Serum testosterone levels were analyzed using RIA / FIA. TCDD doses were high enough to decrease the body weights in each line. Relative organ weights were not affected in line C rats, but VP and SV weights were decreased in line A and line B rats at the highest doses. Cauda epididymal sperm reserves were significantly decreased at 100-1000 μg/kg TCDD in line A and B rats, and at 10-30 μg/kg in line C rats. Serum testosterone levels were decreased in line A (100-1000 μg/kg) and line C (30 μg/kg) rats. As line C rats were most sensitive to many of the studied reproductive endpoints, the resistance alleles Ahr^{hw} and B^{hw} seem to increase the resistance of adult males to these TCDD-induced reproductive defects. (Academy of Finland, Project 42551 and European Comission Contract QLK4-CT-1999-01446)

829 RELATIONSHIP OF TISSUE DOSE OF 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) TO REVERSIBLE BIOLOGICAL RESPONSES.

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Exposure to TCDD and dioxin-like chemicals causes a wide range of adverse effects that can be produced regardless of exposure route and time, but may vary with administered (admin) dose. Dose is uncertain in humans; therefore, appropriate dosemetrics and effects derived from animal data are important for risk assessment. To test the hypothesis: tissue concentration (conc) is a useful dosemetric for reversible biochemical changes, female B6C3F1 mice were acute (ac) or subchronic (sub) dosed with [3H]TCDD at graded doses and times. Livers were removed; TCDD conc, EROD & ACOH (markers for CYP1A1 & 1A2, respectively) were determined. The Hill model was fit to enzymatic data using admin dose, daily dose or tissue conc as dosemetric. When ac or sub EROD or ACOH data were analyzed separately, using total admin or daily dose showed adequate fits to the data ($R>0.75$). When ac and sub EROD or ACOH data were analyzed together, using total admin or daily dose as dosemetric did not provide an adequate description of a Dose-Response (DR) relationship for either endpoint examined. Using TCDD tissue conc as dosemetric resulted in similar DR relationships when ac or sub EROD and ACOH data were analyzed separately or combined. When tissue conc was used as dosemetric, fits resulted in $R>0.88$ and $p<0.0001$ for all data sets analzyed. When the Hill model was fit to: EROD data, ED50s were estimated at 60±10, 20±2.58 and 37±4.5 ng TCDD/g tissue for sub, ac and combined, respectively; to ACOH data, ED50s were estimated at 7.6±0.44, 1.7±0.28 and 4.4±0.78 for sub, ac and combined, respectively. The Hill shape parameter indicates that when tissue dose is used as dosemetric, DR curves are supralinear. While estimates of ED50s were similar, ED50 was slightly higher (2-3 fold) in sub studies compared to ac studies. These studies demonstrate tissue conc is a useful dosemetric for describing DR relationships for reversible biochemical responses to dioxins. (This abstract does not reflect USEPA policy.)

830 17 β-ESTRADIOL METABOLISM AND EROD ACTIVITY IN RAT LIVER AND LUNG AS BIOMARKERS OF CHRONIC EXPOSURE TO TCDD AND RELATED COMPOUNDS.

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The expression of cytochrome P450 1A1 (CYP1A1) and cytochrome P450 1B1 (CYP1B1) represents sensitive biomarkers of exposure to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related halogenated aromatic hydrocarbons (HAHs). CYP1A1 mediated ethoxyresorufin O-deethylase (EROD) and estradiol metabolism were assessed in liver and lung microsomes from female Sprague-Dawley rats following 13 weeks of daily oral (gavage) exposure to TCDD, 2, 3, 4, 7, 8-PeCDF, PCB 126, or PCB 153. Estradiol 4-hydroxylase (4-OHE2) activity, a marker of human CYP1B1 expression, is currently being evaluated as a marker of rat CYP1B1 expression. In addition, estradiol 2-hydroxylase (2-OHE2) activity was measured because of its significant role in estradiol metabolism. TCDD exposure produced a dose related increase in these three activities which, with the exception of 2-OHE2 in liver, were significantly increased at an exposure of 3 ng/kg/d. Tissue-specific differences were noted with lung showing a greater fold induction in 4-OHE2 and 2-OHE2 activities, while liver displayed a greater increase in EROD activity. With the exception of PCB153 (TEF=0), each of the HAHs increased EROD, 4-OHE2, and 2-OHE2 activities in a dose-related manner, which generally supports the current Toxic Equivalency Factors (TEFs) for these compounds. Dose-response relationships following tertiary (TCDD, PeCDF, PCB 126) and binary (PCB 126, PCB 153) exposures at 13 weeks also support the additive activity of these agents, based on the current TEFs for these HAHs. These studies were carried out using tissues provided to us by the National Toxicology Program as part of a series of chronic 2-year rat bioassays examining the relative carcinogenic potencies of dioxin-like compounds. Following completion of the cancer studies, it will be

possible to establish whether tissue-specific biomarker responses are related to the tissue-specific carcinogenic potency of these agents. (Supported in part by NIEHS ES09440, USEPA R825808)

831 ZEBRAFISH FIN REGENERATION AS A MODEL FOR DEVELOPMENTAL TOXICITY OF 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD).

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Adult zebrafish completely regenerate their caudal fins following amputation. Fin regrowth can easily be followed *in vivo* and the new tissue can be used to study developmental processes. 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) is known to cause toxicity through the AhR pathway, binding to AhR allowing it to translocate to the nucleus and bind with ARNT, activating gene transcription of xenobiotic responsive genes. Hif-1α, a protein of the hypoxia responsive pathway, also requires ARNT dimerization for activation of the hypoxia responsive element and transcription of hypoxia genes. The expression of hypoxia regulated genes are essential for wound repair and neovasularization. Our laboratory has been using caudal fin regeneration in zebrafish to investigate molecular mechanisms of TCDD toxicity during development. When adult zebrafish were treated with 70 ppb TCDD (i.p.) and their fins were amputated, there was consistent inhibition of caudal fin regeneration. To determine the regeneration stage blocked by TCDD toxicity, zebrafish caudal fins were amputated and fish were injected at various time points during regeneration. We found that TCDD impaired fin regeneration at multiple stages of regeneration. Effects of TCDD on vasculature regrowth of the fin were studied using transgenic zebrafish that express green fluorescent protein driven by the blood cell specific GATA-1 promoter, allowing us to visualize the functional vasculature. TCDD significantly affects caudal fin vascular structure and function. We propose that TCDD activation of the AhR pathway squelches the Hif-1α pathway, resulting in decreased expression of hypoxia regulated genes, thereby decreasing neovascularization and fin regeneration.

832 INHIBITION OF HUMAN AND RAT CYP1A2 BY TCDD AND DIOXIN-LIKE CHEMICALS.

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The tissue distribution of dioxins is dose-dependent due to hepatic sequestration by CYP1A2, which is an inducible binding protein. Thus the disposition of dioxins is dependent upon the ability to bind and induce CYP1A2. Current risk assessments assume that humans and rats sequester dioxins in a similar manner. The present study examines this assumption by comparing the ability of dioxins to inhibit human and rat CYP1A2 activity. Rat and human CYP1A2 + P450 reductase SUPERSOMES were obtained from GenTest Corporation (Woburn, MA). Methoxyresorufin-O-deethylase (MROD) activity, a prototype substrate, was monitored in human and rat CYP1A2 SUPERSOMES in the presence of varying concentrations of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), 1, 2, 3, 7, 8-pentachloro-*p*-dioxin (PCDD), 2, 3, 4, 7, 8-pentachlorodibenzofuran (4-PeCDF), 2, 3, 7, 8-tetrachlorodibenzofuran (TCDF), 3, 3', 4, 4', 5, 5'-hexachlorobiphenyl (PCB 169), 3, 3', 4, 4', 5-pentachlorobiphenyl (PCB 126), 2, 3, 3', 4, 4', 5-hexachlorobiphenyl (PCB 156), 2, 3', 4, 4', 5-pentachlorobiphenyl (PCB 118), and 2, 3, 3', 4, 4'-pentachlorobiphenyl (PCB 105). Both human and rat CYP1A2 metabolized methoxyresorufin, evidenced by similar K_M values between species (0.10nM human, 0.09nM rat). Rat SUPERSOMES had a slightly higher V_{max} than human SUPERSOMES, 3.5 and 2.4 pmol/min/mg CYP1A2 respectively. Our results demonstrate similar dose-dependent inhibition of human and rat CYP1A2 activity for all chemicals tested. TCDD, TCDF and 4-PeCDF were the most potent inhibitors. These chemicals appear to be mixed type inhibitors, with K_i values of approximately 1-100 μM. Inhibition of CYP1A2 activity by this series of chemicals *in vitro* may explain the lack of correlation between CYP1A2 activity and dioxin exposure in human epidemiology studies (This abstract does not reflect EPA policy.)

833 SUBCHRONIC TOXICITY OF 3, 3', 4, 4'-TETRACHLOROAZOBENZENE(TCAB) ADMINISTERED BY GAVAGE TO HARLAN SPRAGUE-DAWLEY RATS.

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Tetrachloroazobenzene is a by-product formed during the manufacture or environmental degradation of 3, 4-dichloroaniline or its herbicidal derivatives. Because of the structural resemblance of TCAB to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), this study was performed to obtain data for the determination of the potency of TCAB relative to TCDD for a variety of endpoints. Male and female Sprague-Dawley rats (10/sex/group) were administered TCAB (100% pure) in corn oil containing 1% acetone by gavage at dosages of 0(control), 0.1, 0.3, 1, 3, 10, 30 and 100 mg/kg, 5 days/week, for approximately 13 weeks. No treatment-related clinical signs were observed or early deaths occurred during the study. There was a statistically significant treatment-related decrease of less than 8% in mean body weight in all female treatment groups above 0.3 mg/kg at termination. Serum total and free T4 were decreased in all dosage groups with generally undetectable concentrations in the groups above 3 mg/kg; no effects were noted on serum T3 or TSH concentrations. TCAB-related decrease in hemoglobin and increases in alkaline phosphatase and cholesterol were noted in both sexes. Liver CYP1A1, CYP1A2, CYP2B and pulmonary CPY1A1 activities were increased at 0.1 mg/kg and higher in a dose-related manner. Significant effects on P450 activity and thyroid hormones were reported in the lowest dosage (0.1 mg/kg); therefore, a NOEL could not be determined. Dose-dependent induction by TCAB of the expression of cytochromes P450, CYP1A1 and CYP1A2 was compared to the induction by TCDD after 13 weeks of daily dosing under similar study conditions. The daily doses needed for similar levels of induction of these genes was 6 orders of magnitude higher than for TCDD indicating that TCAB is only a weak Ah receptor agonist *in vivo*. (Supported by N01-ES-65406.)

834 COMPARING MIXTURES OF DIOXIN-LIKE AND NON-DIOXIN-LIKE PCBs TO TCDD.

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Environmental exposures to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) always occur as part of a complex mixture. In order to assess the potential risk associated with these exposures, the Toxic Equivalency Factor (TEF) method was developed, and uses toxic equivalents (TEQ) to relate a chemical to TCDD. While this method adequately accounts for the effects of mixtures of TCDD or dioxin-like (DL) chemicals, there are almost always non-dioxin-like (NDL) chemicals present, in particular, the NDL polychlorinated biphenyls (PCBs). To further examine the interactions of dioxin with NDL PCBs, the present study compared the effects on multiple responses of different laboratory-defined mixtures, based on mass ratios found in food, of dioxin and NDL PCBs in both wild type C57BL/6J and CYP1A2 null male and female mice. These chemical groups are: 1) TCDD alone; 2) DL Mix A containing TCDD, 1, 2, 3, 7, 8 Pentachlorodibenzo-*p*-dioxin, 2, 3, 4, 7, 8 Pentachloro-dibenzofuran, PCB 126; 3) NDL Mix B containing PCB 118, PCB 138, PCB 153, PCB 180; and 4) Mix C - a combination of Mixes A and B. The dose levels used were 0.0, 0.001, 0.01, 0.1, 1.0, and 10.0 μg TEQ/kg body weight for Mix A, B and C, and the same plus 100.0 μg /kg for TCDD. No effects were seen in body weight or other tissues, in male or female wildtype or knockout mice. Although we expected an increase in liver weights in Mix B and Mix C, liver weight was increased only in Mix C in both male and female, wildtype and knockout. The effect appears to be synergistic, not additive. No effects were observed in levels of glutathione, a marker of oxidative stress, in either wildtype or knockout, although this is not surprising due to the large pools of glutathione in the body. Based on these findings, it appears that these mixtures have a synergistic effect greater than the sum of the individual congeners, or TCDD alone. (This abstract does not reflect EPA policy. This research was supported by EPA CT902908)

835 EFFECT OF CHLORINATED PHENOTHIAZINES ON OVULATION IN RATS.

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Adult female Sprague-Dawley rats were administered 1mg/kg/day of tetrachlorophenothiazine (TCPT) or its sulfoxide (TCPT-O) in xylene (4ml/kg). Estrous cyclicity of the rats was determined by obtaining vaginal smears for three weeks prior to the beginning of dosing and for two weeks during daily dosing. Similar to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) both phenothianzines had a profound effect on cycling with prolonged periods of diestrous and the loss of proestrous and estrous. The sulfoxide was more potent than the parent compound.

Immature female rats were administered 1 and 2mg/kg/day of TCPT or TCPT-O in xylene (4ml/kg) for 4 days. Equine chronic gonadotropin (eCG; 5IU) was administered 24h after the first dose rate to induce follicular development. The animals were terminated at 72h after eCG injection, ovaries harvested and the number of ova shed counted. There was an insignificant decrease (about 50%) in the number of ova ovulated among the TCPT-treated rats. However, TCPT-O dose-dependently and at the higher dose completely inhibited ovulation. In addition, ethoxyresorufin-O-deethylase (EROD) was determined in the livers of all rats. There was a very slight induction of EROD with both compounds at the higher dose. The structure of TCPT was elucidated by UV-VIS spectroscopy, HPLC, NMR spectroscopy and by GC/MS. Single crystal X-ray spectroscopy provided the final proof for the structure of 1, 3, 7, 9-TCPT.

836 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN INHIBITS TRANSCRIPTIONAL ACTIVITY OF THE 3'α ENHANCER IN LIPOPOLYSACCHARIDE-STIMULATED CH12.LX B-CELLS.

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The B-cell is a sensitive cellular target of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) with the inhibition of the antibody forming cell response being a hallmark endpoint of TCDD toxicity. The actual molecular mechanism responsible for altered B-cell function by TCDD is unclear. Our previous results describing the CH12.LX (AhR-expressing) and BCL-1 (AhR-deficient) B-cell lines have supported an AhR/dioxin-responsive enhancer (DRE)-mediated mechanism for TCDD-induced inhibition of μ heavy chain expression and thus of immunoglobulin (Ig) M secretion. Transcriptional regulation of the Ig heavy chain genes involves several regulatory elements including the 3'α enhancer, which is composed of four regulatory domains. One of these domains, 3'α-hs4, contains a DRE overlapping a κB motif. We have previously demonstrated binding of the AhR nuclear complex and NF-κB proteins to these motifs. In the present studies, a luciferase reporter gene containing only the 3'α-hs4 domain or the full-length 3'α enhancer was transiently transfected into the CH12.LX cells to determine the effect of TCDD and LPS on the activity of these enhancers. The co-treatment of TCDD and LPS had the greatest effect on these enhancers compared to either treatment alone. Interestingly, the co-treatment of TCDD and LPS markedly induced the activity of the 3'α-hs4 domain; whereas, LPS alone induced the full-length 3'α enhancer but this induction was markedly inhibited with the addition of TCDD. Induction of the 3'α-hs4 domain by the co-treatment of TCDD and LPS may negatively regulate the full-length 3'α enhancer leading to inhibition of μ heavy chain expression. (These studies were supported by NIH ES02520 and NIEHS 5F32ES05914-01)

837 NON-COPLANAR POLYCHLORINATED BIPHENYL (PCB) CONGENERS INHIBIT PCB 126-INDUCED ACTIVATION OF THE 3'α-HS4 ENHANCER IN LIPOPOLYSACCHARIDE-STIMULATED CH12.LX B-CELLS.

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Polychlorinated biphenyls (PCB) are ubiquitous contaminants in the environment and many of their toxic effects including inhibition of B-cell differentiation are elicited by binding to the aryl hydrocarbon receptor (AhR). However, non-coplanar, di-ortho-chlorinated PCB congeners have been shown to inhibit AhR agonist-mediated responses, such as inhibition of μ heavy chain expression and immunoglobulin (Ig) M secretion. Transcriptional regulation of the Ig heavy chain gene involves several regulatory elements including the 3'α enhancer, which is composed of four regulatory domains. One of these domains, 3'α-hs4, contains a DRE overlapping a κB motif. In transient transfection experiments, 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) enhanced the activity of a luciferase reporter gene containing the 3'α-hs4 enhancer domain in lipopolysaccharide (LPS)-activated CH12.LX B-cells. The purpose of the present studies was to determine the effect of PCB 126 (3, 3', 4, 4', 5), an AhR agonist, alone or in combination with the non-coplanar PCB 153 (2, 2', 4, 4', 5, 5') or PCB 52 (2, 2', 5, 5') on the activity of the 3'α-hs4 enhancer domain in LPS-activated CH12.LX cells. Similar to TCDD, PCB 126 enhanced the LPS-induced activation of the 3'α-hs4 domain. In addition, PCB 153 and PCB 52 reversed the effect of PCB 126 on 3'α-hs4 activity. Activation of the 3'α-hs4 domain by PCB 126 may alter the normal activation pattern of the 3'α enhancer leading to inhibition of μ heavy chain expression. In addition, PCB 153 and PCB 52 may antagonize the effects of PCB 126 by interfering with the AhR signaling pathway. (These studies were supported by NIEHS ES04911-13 and NIEHS 5F32ES05914-01)

837A INDUCTION OF TELOMERASE BY 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) AND ESTROGEN IN BEWO CELLS.

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In an attempt to clarify the underlying mechanism of tumor progression by TCDD, we studied its effect on telomerase activity. Since telomerase is associated with tumor progression and also controlled by sex steriods, effects of TCDD on this enzyme might be one of the pathways towards its tumorogenic action. We used BeWo cells derived from human placental choriocarcinoma as a model system. Stretch PCR analysis showed that TCDD and estrogen induced telomerase activity in BeWo cells. However, an increase in activity was high with TCDD as compared to an equimolar concentration of estrogen. Co-treatment of TCDD and estrogen resulted in a partial decrease in telomerase activity as compared to TCDD alone. The expression of hTERT, a regulatory subunit of telomerase as determined by RT-PCR, was affected by TCDD alone, E2 alone and TCDD+E2 in a similar manner that to telomerase. Furthermore, the expression of *c-myc*, a positive regulator of hTERT, was also increased by TCDD, suggesting the up-regulation of telomerase was *c-myc* dependent. Co-treatment with E2 and TCDD decreased expression of *c-myc* and was comparable to estrogen alone. Since telomerase activity is often associated with proliferating cells, we performed FACS analysis which revealed that both estrogen and TCDD increased cells from G1 to S phase. In contrast, co-treatment of estrogen and TCDD resulted in a decrease in S phase. Our findings do provide evidence that a low concentration of TCDD(1 and 10 nM) activated telomerase as an estrogen agonist while there is the possibility that TCDD(1 and 10 nM) acts as a partial antagonist in the presence of E2 or that the suppression may be due to a yet unknown mechanism.

838 ALTERED GENE EXPRESSION AND CUTANEOUS TOXICITY.

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Adverse skin reactions can take a variety of forms and be induced by many biologically independent mechanisms. Recently, important progress has been made in defining the molecular bases for skin sensitization, skin irritation and other forms of cutaneous toxicity. In particular the application of transcript profiling and related technologies has allowed the characterization of altered patterns of gene expression that collectively provide important new insights into the relevant toxic mechanisms. Moreover, it is anticipated that the association of novel genes with particular forms of dermal toxicity will yield exciting new opportunities for hazard identification. This proposed Symposium brings together for the first time experts in skin biology and cutaneous toxicity to review how characterization of changes in gene expression are providing new insights into mechanisms of toxicity and opportunities for new approaches to hazard assessment. This Symposium is particularly timely because speakers will be describing the application of transcript profiling and proteomic analysis to monitor changes in gene expression and protein production.

839 INDUCTION OF SKIN SENSITIZATION : GENE EXPRESSION PROFILES.

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An increasingly sophisticated appreciation of the cellular and molecular events associated with skin sensitization, linked with the availability of methods for transcript profiling, provides an opportunity to design alternative approaches to toxicological evaluation based on induced changes in gene expression. A central event in the acquisition of contact allergy is the induction of lymph node cell (LNC) proliferation, a response that forms the basis of the local lymph node assay (LLNA), a method used for the identification and characterization of skin sensitization hazard. We have now examined whether changes in gene expression patterns induced selectively by allergen exposure can be identified using DNA microarray technology. Gene expression changes have been assessed following topical exposure of BALB/c strain mice to the contact allergen 2, 4-dinitrofluorobenzene (DNFB). In parallel investigations, transcript profiling has been performed on human blood derived dendritic cells (DC) cultured in the presence of non-toxic concentrations of

DNFB. Custom DNA microarrays comprising 8734 murine genes (3336 of which are assigned to known genes) or approximately 12500 human genes, each arrayed in duplicate, were utilized. In both cases, the majority of genes was unaffected by treatment with DNFB. Modest DNFB-induced changes in gene expression patterns of human DC were recorded, however, using the current culture conditions, these appear to be largely donor-dependent. Robust changes (up- and down-regulations) in the expression of mRNA for a small number of genes were observed following topical treatment of BALB/c strain mice with DNFB, with onzin and glycosylation-dependent cell adhesion molecule- 1 (GlyCAM-1) being the strongest up- and down-regulated genes, respectively. These genes may provide for robust and relatively sensitive markers of early changes induced by contact allergens.

840 ALTERED KERATINOCYTE GENE EXPRESSION FOLLOWING TREATMENT WITH IRRITANTS.

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In the screening of topic drugs, cosmetics and other chemicals for human use, it should be very important, both from safety and economic point of view, to have biological markers to discriminate irritant and allergic contact dermatitis that have different impact on human health. Due to their anatomical location, keratinocytes are among the first cells to be exposed to various toxicants and, the use of these cells as simplified *in vitro* model to evaluate the potential toxicity of chemicals destined for cutaneous application is amply justified. The identification of new target genes selectively modulated by skin toxicants offers the possibility to develop mechanism-based *in vitro* assay. We have data indicating the possibility to use the mRNA differential display technology (DD-PCR) and DNA macroarray to identify differentially expressed genes following allergen or irritant treatment in commercially available reconstituted human epidermis (EpiDermTM). Results identified adipose differentiation-related (ADRP) protein as up-regulated by both irritant and allergen, and KIAA0368 as selectively up-regulated by contact allergen. ADRP is a 53kDa membrane-associated protein and it is a marker of lipid accumulation, being an ubiquitously expressed lipid storage droplet-associated protein. The induction of ADRP is indicative of increased intracellular lipid deposits, which may herald cell degeneration and maybe also indicative of abnormal keratinization. On the contrary, KIAA0368 is probably an elongation factor involved in protein neosynthesis. It codes for a protein of apparent molecular mass >100kDa, located on chromosome 9 and ubiquitously expressed. Its role in skin irritation or allergy is at present unknown. These data indicate the enormous potential of functional genomic techniques, which allow the identification of genes not immediately connected with the immune response, or even novel genes with unknown functions.

841 MOLECULAR CHANGES IN SKIN FOLLOWING ACUTE DERMAL EXPOSURES WITH JP-8 JET FUEL AND SOLVENTS.

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Many products and chemicals cause irritation when they contact the skin. Whole animal primary irritation testing (Draize test) has been around since 1944 with many modifications and variations. With acute tests, the barrier function of the skin is often disrupted and then the substance is placed in occluded contact with the skin for 24 hours and the responses are graded. Irritancy testing was designed to provide relative irritancy of substances, but these tests don't provide useful information for risk assessments or industrial hygiene. In a real exposure situation, a substance would have to diffuse past the stratum corneum barrier down into the viable tissue to produce irritation. Since chemicals diffuse through the skin at different rates and have different irritant potencies, there is an exposure duration for most substances that would not cause irritation. Only minutes of contact could be safe for some substances but it might take hours or days for other substances to cause irritation. The purpose of these studies, using substances with different irritant properties, is to characterize the biological cascade in the skin that results from an acute irritating event. Ultimately our goal is to develop a biologically based model of irritation that can be used to predict safe exposure durations for a wide variety of compounds. We exposed the backs of rats to jet fuel, m-xylene, d-limonene and sodium lauryl sulfate for one hour and investigated the temporal changes in interlukin-1 alpha levels, inducible nitric oxide synthase levels and nitric oxide production. We also measured the time course of lipid peroxidation, oxidative stress and low molecular weight DNA levels in response to these substances. Traditional histopathology and immunohistochemistry were also used to compare response of the skin to the fuel and solvents. We found that the parameters in the irritant cascade that we investigated responded differently depending on the degree of irritancy of each chemical. Further work will refine our preliminary understanding of this acute irritant cascade.

842 ALTERED GENE EXPRESSION IN KERATINOCYTES FOLLOWING ARSENIC EXPOSURE.

D. R. Germolec. *Environmental Immunology, NIEHS, Research Triangle Park, NC.*

Previous studies in our laboratory have suggested that arsenic modulates neoplastic disease in the skin by inducing overexpression of genes encoding specific cytokines and growth factors. We have completed *in vitro* studies which compare altered cytokine gene expression following exposure to either ArsenicIII and ArsenicV or their monomethyl and dimethyl metabolites in normal human epidermal keratinocyte (NHEK) cultures and have found that methylated arsenicals may be as potent as their inorganic counterparts. Microarray studies using NHEK indicate that short-term, non-toxic arsenic-exposure results in the modulation of multiple genes from several classes (e.g., oxidative stress, glutathione metabolism, heat shock/stress response, cell proliferation and DNA damage). Gene expression changes have been validated *via* RPA and northern blotting. Additional microarray studies have examined similar/dissimilar gene expression profiles in NHEK and HaCaT keratinocytes (an immortalized cell line commonly used in arsenic, oxidative stress, and epidermal research) following arsenic exposure. Pathway mapping, FACs analysis and viability studies also have been performed and the correlation between altered gene expression and physiological effects (e.g., cell cycle arrest, DNA damage, elevated cell proliferatory responses, etc.) will be presented.

843 OVEREXPRESSION OF MOUSE HOMOLOGUE OF HOS UBIQUITIN LIGASE RECEPTOR IN SKIN TUMORS IMPLICATION IN CONSTITUTIVE ACTIVATION OF NF-κB.

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The mouse skin model of multistage carcinogenesis is an excellent model to study the critical role of specific gene expression in the induction of squamous cell carcinomas. We have found that the function of NF-κB is altered during the induction of skin cancer. NF-κB transcription factor is activated upon ubiquitination and subsequent proteolysis of its inhibitor IκB. The phosphorylation-dependent ubiquitination is mediated by SCF E3 ubiquitin ligase. We identified a novel murine F-box/WD40 repeat-containing protein, mHOS (a homologue of HOS/βTrCP2). mHOS efficiently binds Skp1 protein (a "core" component of SCF ubiquitin ligase), and phosphorylated Iκβa. We found that mHOS associates with SCF-ROC1 E3 ubiquitin ligase activity. We have also observed that mHOS is overexpressed in chemically-induced mouse skin tumors, and its overexpression (but not accelerated IκB phosphorylation) coincides with the accelerated degradation of IκB *in vivo*. The role of mHOS in the constitutive activation of NF-κB in skin carcinogenesis will be discussed.

844 DEFINING THE CELLULAR AND MOLECULAR MECHANISMS OF TOXICANT ACTION IN THE TESTIS.

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Despite the association of environmental toxicant exposure and testicular injury, there is little mechanistic data of the molecular and cellular pathways involved. This symposium is focused on the identification of novel molecular and cellular pathways that modulate the response to testicular toxicants. The recently discovered G-protein coupled receptor family member, flamingo, mediates cell-cell adhesion and has been identified in the testis. A specific subset of flamingo homologs exhibits a reduced cell-specific expression at early time points after phthalate exposure suggesting that they may be proximal phthalate targets. Observations of the newly identified death receptors (DR4, 5 & 6) in the testis and differential cellular changes in their expression after phthalate exposure suggest that they may act as surrogates or in concert with the widely described Fas-signaling pathway in the initiation of germ cell apoptosis. A variety of exposures in rodents (radiation, chemotherapeutics, toxicants) results in a persistent post-exposure testicular atrophy. Hormonal manipulations that lower testosterone and serum FSH levels allow for a re-initiation of spermatogonial development. Recent investigation of additional models of persistent atrophy such as knockout mice, the aged Brown Norway rat, EDS-induced Leydig cell deficient rat, and primates, have broadened insight into the mechanisms responsible for persistent atrophy. cDNA arrays are a useful technique for examining changes in mRNA expression. A custom mouse testis

cDNA array has been developed that contains 950 genes. cDNA array analysis of testis mRNA from mice exposed to bromochloroacetic acid (BCA), a drinking water disinfectant, shows an alteration in 42 of 950 genes. Of these, a specific set of 10 genes was identified that represents proteins involved in cell adhesion complexes. This strengthens the findings that BCA acts by disrupting spermiogenesis and provides new insights into the cellular and molecular mechanisms by which BCA acts.

845 PURSUIT OF THE TESTIS PHTHALATE TARGET: A NOVEL CADHERIN IS AN EARLY RESPONDER.

K. Johnson, S. Thompson and K. Boekelheide. *Pathology and Lab. Medicine, Brown University Medical School, Providence, RI.*

Phthalates are environmental pollutants found in soft plastics, and human exposure levels are significant. Phthalates target the testis, with toxicity features including early alterations in Sertoli cell morphology and biochemistry, disruption of Sertoli-germ cell adhesive contact, and altered Sertoli cell G protein-stimulated cAMP generation. These endpoints indicate that a Sertoli-germ cell adhesion complex and a G protein coupled receptor (GPCR) signaling pathway are early phthalate targets. Recently, GPCR family members termed flamingos were discovered which mediate cell-cell adhesion, likely *via* extracellular cadherin domains. Thus, flamingos appear to couple cell-cell adhesion to the stimulation of G protein-based signaling, making them attractive early phthalate targets. Using a degenerate RT-PCR cloning strategy, we discovered that all three flamingo homologs (1, 2, and 3) are expressed in rat testis with unique, cell-specific expression patterns. By analyzing flamingo mRNA expression in postnatal testis, flamingo1 and 3 are likely Sertoli cell products while flamingo2 a germ cell product. To determine if flamingo biology is affected by phthalate exposure, the testis mRNA expression of these flamingos was examined by semi-quantitative RT-PCR following exposure of 28 day old rats by gavage to 1 gm/kg mono-(2-ethylhexyl) phthalate. Significantly, the mRNA expression of flamingo1 and 2was reduced to 70% of control within 3 hrs with additional declines at later time points (35-50% of control at 6 hrs). However, the effect of phthalate was specific to flamingo 1 and 2 GPCRs; the mRNA level of another Sertoli cell GPCR (the FSH receptor) was reduced somewhat at 3 hrs but recovered at 6 hrs post-exposure, while flamingo3 mRNA levels were elevated at 6 hours. These data indicate that flamingo1 and 2 respond early after phthalate exposure and lead to a model of testicular phthalate toxicity in which a flamingo-based Sertoli-germ cell adhesion/signaling complex is a proximal phthalate target, leading to germ cell detachment and apoptosis.

846 NOT SO FAS: EVIDENCE FOR THE DIFFERENTIAL INVOLVEMENT OF FAS-INDEPENDENT SIGNALING PATHWAYS IN THE TESTIS.

J. H. Richburg. *College of Pharmacy, The University of Texas at Austin, Austin, TX.*

The Fas-FasL signaling system is proposed to be critical in the regulation of testicular germ cell apoptosis and maintenance of spermatogenesis. The Fas-signaling system has been implicated in the initiation of germ cell apoptosis after exposure to the Sertoli cell toxicant mono-(2-ethylhexyl) phthalate (MEHP). Interestingly, *gld* mice, which express a mutant form of FasL, are fertile and display normal spermatogenesis. In addition, a limited protection against MEHP-induced germ cell apoptosis was observed in *gld* mice. To evaluate the participation of non-Fas death receptor signaling systems in the testis, we examined the expression of the newly described death receptors (DR 4, 5 and 6) in both C57BL6 (B6) and *gld* mice after MEHP exposure (1 g/kg). By western blot analysis, each of the DR's was present in B6 and *gld* mouse testis. A comparison of the pattern of protein expression of Fas to that of the DR's in B6 mice after MEHP exposure revealed that Fas, DR4 and DR5 show similar patterns of expression whereas DR6 has a unique expression pattern. DR6 expression in *gld* mice was also strikingly different from that in B6 mice with an early increase in protein expression (3 h) that remained for the duration of MEHP exposure. The delayed induction of DR6 after MEHP exposure in the B6 testis suggests that this receptor may be activated as a secondary response in B6 germ cells to decreased hormonal, nutritive or physical support that results from MEHP-induced Sertoli cell injury. The early induction of DR6 in *gld* mice implies that the mechanism that leads to the induction of DR6 is already primed. The evaluation of NF-κB in the *gld* testis shows an early increase in NF-κB DNA binding similar to the changes in DR6 expression seen in these mice. Taken together, these findings indicate that the DR4, 5 or 6 systems in mice may be functioning in parallel with the Fas system to regulate germ cell apoptosis and/or that one or more of these DR's may act as a surrogate for the Fas system when it is either overwhelmed or dysfunctional.(Supported by NIEHS ES09145 & ES07784)

847 REVERSAL OF TOXICANT-INDUCED TESTICULAR ATROPHY: MODELS AND MECHANISMS.

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In rodents, severe toxicant-induced testicular injury results in a persistent post-exposure atrophy with ongoing proliferation of stem germ cells and apoptosis of their progeny. The block to further spermatogonial development is relieved by hormonal manipulations that lower testicular testosterone and serum FSH levels. In the rat it appears that both the testicular testosterone and FSH levels are responsible for inhibiting spermatogonial development, whereas in a similar model of spontaneous spermatogenic failure, jsd mutant mice, it appears to be only testosterone. Any androgen appears to be inhibitory and acts through the androgen receptor. In some cases, even a transient suppression of testosterone can result in maintenance or reinitiation of spermatogonial development for a prolonged period to restore complete spermatogenesis and fertility. However, therapy with the toxicant ethane dimethane sulphonate, which selectively ablates testosterone-producing Leydig cells, suppresses both serum testosterone and testicular testosterone yet fails to stimulate a reversal of 2, 5-hexanedione-induced injury, regardless of whether it is administered alone or in combination with GnRH agonist therapy. These studies suggest that paracrine-acting factors produced by Leydig cells may also be required for the reversal of testis injury. This paradigm of induction of otherwise "irreversible" injury, followed by hormonal manipulations to maintain spermatogenesis, is currently being tested in a non-human primate model. The results of this primate study will be discussed along with insights into the mechanism of atrophy reversal derived from ongoing investigations of rodent models.

848 GENOMIC ANALYSIS OF THE TESTICULAR TOXICITY OF HALOACETIC ACIDS.

D. J. Dix and J. C. Rockett. *Reproductive Toxicology Division, USEPA, Research Triangle Park, NC.*

Gene expression profiles from testes of mice exposed to effective doses of reproductive toxicants can be used to identify genes and gene networks critical to male fertility, infer toxicant mode of action, and understand gene-environment interactions. A custom mouse cDNA microarray interrogating 950 genes was used to analyze gene expression in the testes of mice exposed to a haloactetic acid (HA). HAs are unintended byproducts of drinking water disinfection. Bromochloroacetic acid (BCA) is a commonly occurring HA that acts as a reproductive toxicant in male rodents, targeting sperm and spermatids. Adult C57BL/6N male mice were dosed *via* gavage with 0, 8, 24, 72 or 216 mg/kg of BCA once daily for 14 days. The mice were then used in a 40-day sequential breeding assay to determine if BCA negatively affects reproductive performance by targeting a particular phase of spermatogenesis. Decreased fecundity and infertility was observed during the first 10 days of the breeding assay, indicating that BCA was affecting post-meiotic spermatogenesis. Histological analysis of testes harvested at the end of dosing revealed spermatid retention and abnormal residual bodies, consistent with disruption of spermiation (the process of spermatid separation from Sertoli cells). DNA array analysis of these testes indicated that expression of 42 of the 950 genes interrogated was significantly altered by the higher doses of BCA. Of the 42 altered genes, 10 were cell adhesion genes, 7 stress response genes, and 4 steroid metabolism genes. These 10 cell adhesion-signaling genes included vinculin, a catenin, an integrin and tubulin. These families of proteins are components of or associated with the ectoplasmic specializations (ES) which form cell-cell junctions between Sertoli cells and between Sertolis and spermatids. Thus the gene expression results suggest that BCA exposure disrupts normal ES function and signaling cascades regulating sperm release. Further studies to examine the dose-responsiveness of these effects on gene expression are underway. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy)

849 DRUG-INDUCED HUMAN HEPATOTOXICITY: PREDICTIVE STRATEGIES.

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Clinical studies have been the pivotal step of drug development in modern medicine. Despite extensive and thorough evaluation in preclinical species, hepatotoxicity is the most common cause for drug failures during clinical trials. Our inability to adequately predict adverse events in patients following the administration of therapeutic compounds is an industry-wide enigma. The enhancement of our knowledge-based understanding of the adverse responses arising from the peculiarities of genetic diversity will greatly improve the robustness of our prediction. The goal of this symposium is to present new developments in our understanding of human

liver toxicity following the administration of pharmaceuticals. A broad range of fruitful avenues worthy of pursuit includes advances in pharmacogenomics, virtual space, and predictive systems utilizing emerging technology platforms.

850 DRUG-INDUCED LIVER INJURY IN MAN: PHARMACOGENOMICS AND PREDICTIVE SYSTEMS.

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Most drugs that are recognized as capable of causing severe liver injury in man cause no liver injury at all in most treated patients. That is, only a small subset of treated patients are susceptible to the injury. The basis for this idiosyncrasy is widely believed to largely be genetic. As genomic DNA is now routinely collected in drug trials, it is possible to test hypotheses concerning specific gene mutations by searching for these mutations in patients with and without signs of liver toxicity, usually serum alanine aminotransferase [ALT] elevations. A problem with this mutation specific approach is that target genes are rarely known, and it is likely that toxicity reflects mutations in multiple genes simultaneously present in the susceptible individual. An alternate approach is to simultaneously screen the genomic DNA pools for many single nucleotide polymorphisms (SNPs) simultaneously to identify regions of the genome that may contain susceptibility genes. When associations are found, the specific genes and mutations can theoretically be identified. A problem with this approach includes the large number of samples that must be analyzed and the current costs of this analysis. It should also be noted that with many drugs, most patients with ALT elevations in clinical trials will have reversal of the elevations, and hence resolution of the liver injury, even if continued on drug. The "adaptation" may involve multiple mechanisms, including immune tolerance. The subset of patients who develop clinically important liver injury may be those with genetic mutations that prevent them from adapting to the initial injury. Since most patients in clinical trials have treatment discontinued when serum ALT rises significantly, it is not possible to identify the poor adapters. This issue may only be addressable after creation of a nation-wide bank of DNA obtained from patients with severe liver injury due to specific drugs.

851 HEPATOTOXICITY: VIEWS FROM VIRTUAL SPACE.

G. M. Pearl. *Discovery Safety Optimization, Bristol-Myers Squibb, Hopewell, NJ.* Sponsor: S. Durham.

Currently, hepatotoxicity is the most common safety liability responsible for the attrition of drug candidates in the discovery and development process, including black box warnings issued on marketed pharmaceuticals. Despite the importance of this liability, there are no commercially-available predictive computational models for hepatotoxicity. We have established computational modeling algorithms for other safety liabilities that have had a favorable impact on the drug discovery process, especially when these models are used as a sentinel filter for predicting common drug safety liabilities. We are currently developing a predictive hepatotoxicity model that concentrates on the subset of chemical space related to pharmaceuticals. This model is based upon the various statistical algorithms that utilize a central structure activity relationship (SAR/QSAR) scaffold. The standard statistical toolkit, including recursive partitioning, principle component analysis, and neural networks, are especially useful in the development of these models. This presentation will focus on the global strategies, tactical approaches, and infrastructure requirements necessary to establish a robust virtual model of hepatotoxicity.

852 APPLICATION OF EMERGING TECHNOLOGIES FOR THE IDENTIFICATION OF "FINGERPRINTS" OF HUMAN HEPATOTOXICITY.

D. M. Dambach. *Discovery Safety Optimization, Bristol-Myers Squibb, Princeton, NJ.*

Hepatotoxicity remains the most common cause for drug failures during clinical trials. This is due, in part, to inherent physiological differences between humans and pre-clinical rodent species leading to inadequate prediction of adverse responses in humans. To address this issue, there is a need to develop robust assays and identify biomarkers which are predictive of human hepatotoxicity. The ideal biomarker should be utilized throughout the discovery and development periods to select the safest drugs. Predictive assays, suitable biomarkers, and the determination of the mechanisms of toxicity for compound classes are the new focus areas within drug safety evaluation. An ever-expanding armamentarium of new technologies is being utilized to help identify macromolecular endpoints as potential surrogates of toxicity. The profiles very useful for evaluation include the transcriptome, the proteome, and the metabonome. We applied transcriptional and proteomic analyses in parallel to compare platform technologies and identify potential biomarkers of PPAR-gamma agonist-induced hepatotoxicity. Comparative analysis of oligo- *versus* cDNA-based arrays, and surface enhanced laser desorption ionization (SELDI) *versus* LC-LC/MS protein identification methods proved that the benefits of these platforms were additive. The application of both transcriptional and proteomic analyses expanded the potentially useful endpoints for toxicity biomarker identification.

853 COMPARATIVE DRUG-INDUCED HEPATOTOXICITY EXPRESSION PROFILES IN RODENTS AND HUMANS.

J. A. Rininger. *CuraGen Corporation, New Haven, CT.*

The ultimate goal of gene expression and toxicogenomics analyses is to improve the efficiency of drug development by prioritization of lead compounds. Unfortunately, a continuing limitation is the relevance of gene expression profiles derived from animal model systems to human toxicity and efficacy. The commercial availability of primary human hepatocytes represents a promising means of bridging this gap. However, the use of human hepatocytes in toxicogenomic studies is challenged by variables associated with human donors such as age, gender, ethnic background, and medical history. To evaluate the reproducibility of gene expression studies with primary human hepatocytes, our laboratories have employed GeneCalling® and RTQ-PCR to generate and compare gene expression profiles from different human donor cells treated with PPAR-gamma agonists. This presentation will also compare the gene expression profiles of PPAR-gamma agonists in primary human hepatocytes to those observed primary rat hepatocytes and rat liver *in vivo*. Primary human hepatocytes were non-responsive to prototypical peroxisome proliferator-modulated genes observed in the rodent models. In addition, human hepatocytes showed reproducibility of response in target genes that suggest a different mechanism of action for the fibrate drug class.

854 FACTORS THAT AFFECT THE IMPACT OF DELIVERED DOSE OF INHALED MEDICINES: AN UPDATE OF THE ISSUES.

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There have been rapid advances in the use of the respiratory tract as a route of administration for aerosolized medicines to combat systemic disease *via* the pulmonary vasculature, or to treat lung specific disorders such as asthma, chronic obstructive pulmonary disease or cystic fibrosis. Inhaled pharmaceuticals offer a painless and reasonably convenient technology for the treatment of several life-threatening diseases, but the consistent daily delivery of the prescribed dose of medication is critically dependent upon physical and physiological factors. Exacting aerosol engineering is needed for metered dose inhalers and dry powder inhalers to generate regulated amounts of medicines of defined particle size distributions to reach targeted sites within the respiratory tract. Physiological factors that greatly influence the quantity of drug reaching these sites are breathing patterns, volumes and flows which vary among individuals with age (pediatric vs adult) and extent of pre-existing disease, which may also significantly alter absorption. Following deposition of the inhaled aerosol at the desired site within the respiratory tract, subsequent retention (for pulmonary disease) and bioavailability (for treatment of systemic disease) of the drug are critical factors in minimizing adverse reactions and obtaining maximum efficacy. This workshop should provide the audience with an understanding of new developments in the technology for the delivery of drugs by the respiratory tract administration, and reviews of updated modeling approaches for deposition of pharmaceuticals and current physiological factors that may affect the deposition and bioavailability of these intentionally biologically active, potent compounds. Finally, clinical aspects of inhaled therapeutic aerosols, including current treatments for various disease states, the side effects (or lack of) of inhaled steroids, receptors and targets for maximum therapeutic effect, and innovative therapies using the respiratory system as the portal of entry, will be discussed.

855 INNOVATIONS IN DEVICES AND ENGINEERED POWDERS FOR THE DELIVERY OF INHALEABLE MEDICATIONS.

A. R. Clark. *Inhale Therapeutic Systems Inc., San Carlos, CA.* Sponsor: B. Stuart.

The first pressurized metered dose inhalers (pMDIs) and single dose dry powder inhalers were introduced nearly 50 years ago. Their introduction marked a new era in the treatment of respiratory diseases. However, these early devices suffer from relatively poor efficiency and reproducibility, typically delivering only 5 to 20% of the

nominal dose to the lungs. While adequate for an inexpensive molecule with a wide therapeutic index, improvements were needed if the lung was going to fulfill its full potential as both a topical and systemic delivery site. Over the last two decades improvements in efficiency and compliance have been sought in both device design and particle/formulation engineering. Initially "add-on" devices for pMDIs and multidose versions of dry powder inhalers were developed. Recently, however, fundamentally new device and formulation technologies have emerged. Active devices, which use an internal energy source to supply the energy for aerosol generation, have been developed by a number of companies. Powder technologies, using novel excipients and/or particle morphology to improve powder properties, have also been developed for use with conventional device technologies. While none of these new technologies have reached the market yet they promise improved delivery efficiency, 60-70% of the nominal dose reaching the lungs, and improved reproducibility and compliance.

856 USING MODELS THAT PREDICT INHALED AEROSOL DEPOSITION.

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Physical properties of the aerosol and physiological factors of individual subjects affect the amount of inhaled medicine to reach targeted sites within the respiratory tract. Some, but not all, of these factors are included in existing models that predict deposition of inhaled material. These models broadly fall into four basic categories: empirical models, deterministic-(single or typical)-path models, deterministic-multiple-path models, and stochastic models. All of the models incorporate to some extent physiological states in the population. Deterministic and stochastic models incorporate physical properties of the aerosols and mechanisms of airflow and deposition, while empirical models for the most part do not. All models predict total deposition in the respiratory tract, but only a few multiple-path models predict lobar deposition or depositions relevant to respiratory tract tomography. Stochastic models provide the greatest flexibility in representing the impact of intersubject variability on the deposition of inhaled medicinal aerosols. Software programs for some empirical and deterministic deposition models are available for user calculations on a personal computer. Sufficient information on other empirical and deterministic models is available for user programming, while several deterministic and stochastic models are available only at the sites where they were first created. Since most models are compared against a common inhalation exposure database, the predictions of aerosol deposition are similar for standard subject size and breathing rate but begin to differ significantly for other physiological states. Because of their availability and continued refinement in the incorporation of computational fluid dynamics, deposition models will play a key role in setting design criteria for the next generation of inhalers.

857 PHYSIOLOGICAL FACTORS AFFECTING INHALED AEROSOL DEPOSITION.

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The deposition patterns of inhaled particles show enormous variability both among individuals and for a given individual at different times. Theoretical and empirical models indicate that variations in respiratory tract anatomy and breathing patterns are major contributors to this variability. The primary purpose of this presentation is to discuss how age and gender influence airway anatomy and ventilation, and thus affect the deposition of inhaled particles. Postnatally, the respiratory tract undergoes significant remodeling and enlargement for about 15 years. During this period, airway sizes correlate with body size, and changes in oxygen demand produce changes in respiratory frequency and tidal volume. Such anatomical and ventilatory changes after early development appear to be more closely correlated to body size than age per se. Respiratory tract growth appears to cease when general body growth stops. During normal senescence, the changes in anatomy and ventilation are not very well understood, but include an increase in anatomical dead space. Gender-related differences in airway anatomy are largely related to body size effects, but gender-specific characteristics of the upper airways are known to exist. Clinical measurements of aerosol deposition in children and women are consistent with predictions made by using the existing information on anatomy and ventilation. Information on particle deposition in old age is difficult to find. Even when age, gender, body size and ventilation are known, aerosol deposition patterns are variable. It is apparent that additional details relating to respiratory tract anatomy and ventilation must be explored in order to predict the aerosol deposition pattern for any specific individual.

858 THE EFFECT OF PARTICLE SIZE ON THE RETENTION AND DISPOSITION OF INHALED MEDICATIONS.

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The aerodynamic behavior of solid dry particle or liquid droplet aerosols of inhaled medicines produced from nebulizers, metered dose inhalers (MDI's) or dry powder inhalers (DPI's) determines their deposition site within the respiratory tract of the patient. However, the deposition site environment, including airway or alveolar lining fluids, and availability and viability of pulmonary macrophages, as well as the particle's size and surface area, determine the subsequent retention, disposition and efficacy of the inhaled aerosolized drug. The effectiveness of the inhaled medication in the control of asthma and chronic obstructive pulmonary disease will depend upon its retention time at the target site, with limited systemic absorption. Here the role of drug carrier materials can be important. Conversely, the rapidly expanding use of the respiratory tract as a route for treatment of systemic diseases such as osteoporosis, diabetes or hepatitis C is based upon the rapid dissolution of small aerodynamic size inhaled drug particles deposited in the alveolar lung, comprising 100 square meters of surface in healthy adults, plus intimate contact with the extensive pulmonary vasculature. Recently available data and approaches in predictive modeling of pulmonary retention, dissolution and cell mediated clearance will be examined as they apply to the efficacy of inhaled medications.

859 AEROSOL THERAPY FOR PULMONARY AND SYSTEMIC DISEASE: FROM ASTHMA TO DIABETES AND BEYOND.

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The past 20 years has seen important advances in treating pulmonary and systemic disease with aerosols. Aerosols provide needleless administration, IV-like onset of action, absent first-pass metabolism and a greatly improved therapeutic index. Asthma and chronic obstructive pulmonary disease (COPD) are syndromes that afflict about 30, 000, 000 Americans for which inhaled bronchodilators (beta-agonists; anticholinergics) and corticosteroids (mainly for asthma control) are safe, efficacious, and cost effective in adults and children. Administered by metered dose inhalers (MDIs), dry powder inhalers (DPIs) and small volume nebulizers (SVNs) they are likely to remain the mainstay of therapy for many years. Chronic Pseudomonas aeruginosa infections in cystic fibrosis (CF) and non-CF bronchiectasis are treated with high dose inhaled aminoglycosides (also ceftazidime or peptide antibiotics) to improve the therapeutic ratio, increase bacterial killing and minimize bacterial resistance. Antibiotics and inhaled rhDN-ase, mannitol or hypertonic saline mobilize secretions and improve pulmonary function. In immuno-suppressed patients, pulmonary fungal infections, particularly with Pneumocystis carinii and Aspergillus fumigatus, are frequently lethal. For chronic prophylaxis, nebulized pentamidine and amphotericin B, respectively, are safe and effective. Aerosol therapy may also be used for systemic disease. Instilled/aerosolized small molecules (e.g., morphine for pain in pulmonary edema) achieve rapid IV-like therapy. Macromolecular (proteins/peptides) absorption using aerodynamically small particles (< 3um) deposited in the alveoli has enabled commercial development of inhaleable insulin. Additional inhaleable macromolecules under active development include growth hormone, parathormone, antiproteases, leuprolide, interferons, and many others. Future inhaleables will probably include viral and bacterial vaccines, lung cancer prophylaxis/therapy with modified adenovirus "scavengers" and/or chemotherapy as well as IL-2 and GM-CSF. In the coming decade, dozens of important medications will be administered by inhalation and provide needleless "therapy without tears".

860 MODELING OF POPULATION VARIABILITY.

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One key aspect in toxicological risk assessment is the variability in the population in response to a toxicant. In essence, this variability determines the shape of the dose-response curve (at the population level). Knowledge of the shape of the curve, especially in the lower dose range, is helpful in defining assessment (uncertainty/safety) factors. Such knowledge may reduce the need for such factors, and is helpful to successful risk assessment. Variabilty may be predicted in pharmacokinetic, pharmacodynamic, and other biomathematic models e.g., by Monte Carlo techniques, where the output is generated from repeated simulations using parameter distributions, rather than from single simulation with single parameter values. On the other hand, variabilty of population parameters within a specific model may be estimated by mixed effects modeling or hierarchical population modeling, e.g., in a Bayesian statistical framework using Markov chain Monte

Carlo simulations. The objective of this workshop is to provide the state-of-the art of different approaches to estimate the population variability in model parameters and to predict population variability in toxicity.

861 DATA-DERIVED REPLACEMENT OF DEFAULT UNCERTAINTY FACTOR.

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Uncertainty (or safety) factors in noncancer Dose-Response assessment have been used as a rough indication of the variability in the population Dose-Response curve of a toxicant. In essence, these factors divide a NOAEL (a point on the Dose-Response curve of low but generally unknown response) to estimate a subthreshold dose (i.e., zero response). Different shapes of Dose-Response curves could be used to adjust the value of these uncertainty factors, but generally are not. This presentation will focus on the history and scientific basis of uncertainty factors, newer methods to estimate uncertainty factors based on compound specific pharmacokinetic and pharmacodynamic data, and Monte Carlo analyses in estimating the probability of uncertainty factors. Each of these methods will be related to population variability to the toxicity of a chemical as exemplified by the Dose-Response curves of different chemicals.

862 MIXED EFFECTS MODELING OF TOXICOKINETIC DATA.

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The analysis of sparse pharmacokinetic data in clinical studies has become known as the population approach and is now widely used in drug development. The population approach has not been applied as extensively to pre-clinical pharmacokinetic data, often gathered in toxicokinetic studies. Toxicokinetics is the assessment of systemic exposure in toxicity studies, in which pharmacokinetic data are generated, either as an integral component in the conduct of the nonclinical toxicity studies or in specially designed supportive studies, in order to assess systemic exposure. The data may be used in the interpretation of toxicity findings and contribute to the assessment of the relevance of these findings to clinical safety. Data may be obtained from all animals in a toxicity study, in representative subgroups, in satellite groups or in separate studies. Applying a mixed effects modelling approach in toxicokinetics offers many advantages over the current approach of having satellite groups. Sparse samples for measuring drug/metabolite concentration are collected in all main animals in the majority of studies where toxicological findings are obtained. Such sampling is unlikely to distress the animals, disturb the conduct of a toxicological study or affect the outcome of the study. Many of the outcome measures in toxicological studies are categorical in nature. For example, lesions may be scored on a one to four scale, from none to severe. The analysis of such data is usually carried out using a general mixed modelling approach. In this presentation data arising from a toxicokinetic experiment and a clinical study with pharmacokinetic data will be used to illustrate the principles of general mixed effects modelling, particularly the analysis of sparse pharmacokinetic and pharmacodynamic data.

863 BAYESIAN APPROACHES IN RISK CHARACTERIZATION.

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Complex physiological models are increasingly used for exploring hypotheses about mechanisms of toxicity and for risk assessment. Such models cannot and should not be simply fitted to data, but they still deserve an appropriate statistical treatment (e.g., to help decide about the model validity, choose between competing models, or dissociate uncertainty from variability in model parameters and predictions). Recent developments in computational statistics, particularly in a Bayesian framework, offer new scientific tools to tackle the problem. We first present an overview of the basic concepts needed to implement these tools in toxicology: prior *vs.* posterior distributions, data likelihood, information flow, hierarchical population modeling, and Markov chain Monte Carlo simulations. Examples of recent applications are given to illustrate the power of these methods for model calibration, model checking and validation, uncertainty analysis, study of variability, and optimization of experimental design. It is argued that these tools will clear the way for further progress on model development, and for an improved treatment of sparse biomarker data in occupationally or environmentally exposed human populations.

864 ESTIMATION OF INDIVIDUAL VARIABILITY BY POPULATION PBPK MARKOV-CHAIN MONTE CARLO ANALYSIS.

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The health hazard of a pollutant is in general more closely related to internal exposure to chemical, i.e. target dose, than to external exposure. Thus, population variability in target dose is of great importance in risk assessment. We have used a three-step approach to assess inter-individual variability in target dose and health hazard. In a first step, we retrieve raw data from controlled inhalation exposure of human volunteers to some volatiles (methyl chloride, dichloromethane, toluene, and styrene). We then subject these data to a Bayesian analysis by Markov-chain Monte Carlo (MCMC) simulations within a population Physiologically-Based Pharmacokinetic (PBPK) model. The Bayesian analysis makes it possible to merge prior knowledge on model parameters with the toxicokinetic information embedded in the experimental data and yields posterior estimates of the mean and distribution of the model parameters at the individual (representing measurement error and intra-individual variability) as well as the population level (representing inter-individual variability). In a second step, we calculate the population variability in target dose by regular Monte Carlo simulations, combining the results from the first step with population data on age, gender, body build, metabolic genotype, etc, in a population PBPK model. This step has been carried out for methyl chloride (using total amount metabolized as target dose surrogate) and dichloromethane (amount metabolized *via* the GST pathway). In a third step we calculate the variability in health effect or risk as a function of target dose. So far, we have done this for dichloromethane, by applying the linearized multistage model on the target dose calculated as above. Obviously, this step requires more quantitative knowledge about the relationship between target dose and effect (toxicodynamics) than is presently available for most chemicals. The project was funded by the Swedish council for working life and social research.

865 IMMUNE SUPPRESSION BY TCDD REQUIRES AH RECEPTOR EXPRESSION IN T LYMPHOCYTES.

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The cellular basis for the potent suppression of T cell-mediated immune responses in mice following exposure to TCDD is not understood. Although activation of the AhR is required, the specific AhR+ cells that transduce the suppression have been difficult to identify *in vivo*. The recent availability of AhR-/- mutant mice provides novel ways to investigate the direct targets of TCDD. We used an *in vivo* graft *versus* host (GVH) model of T cell immunity to address the direct AhR-dependent effects of TCDD in T cells. In this model, T cells from C57Bl/6 (B6) mice are injected into B6xDBA/2(D2) F1 host mice. The B6 T cells recognize the MHC disparity of the B6D2 host cells, leading to the generation of a CD4+ T cell-dependent CD8+ CTL response that results in loss of body weight by the host. By comparing the ability of TCDD to suppress the GVH response of T cells derived from AhR+/+ and AhR-/- mice, the importance of the AhR in T cells could be determined. We found that the CTL response of T cells from AhR+/+ mice was suppressed when host mice were treated with 15 µg/kg TCDD, and the host mice did not lose body weight. However, when grafted T cells came from AhR-/- mice, there was no suppression of the CTL response by TCDD, and the host animals lost significant body weight. T cells derived from AhR+/+ and AhR-/- mice generated comparable CTL responses in the absence of TCDD, indicating that the absence of the AhR did not alter the innate responsiveness of the T cells. To determine if AhR-mediated effects of TCDD were selective for CD4+ or CD8+ cells, T cells from AhR+/+ and AhR-/- mice were separated into CD4+ and CD8+ subsets, intermixed in various combinations, and injected into host mice. Results showed that the AhR must be expressed in both T cell subsets to have full suppression of the GVH response by TCDD. Future studies will exploit the GVH model using a variety of mutant T cells to directly assess the role of various signaling pathways involved in AhR-mediated T cell dysfunction. (Supported by P01 ES00040 and OSU Unsponsored Research Fund)

866 EXPOSURE TO TCDD SUPPRESSES THE EXPANSION OF ANTIGEN-SPECIFIC T CELLS DURING INFECTION WITH INFLUENZA VIRUS.

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One of the most sensitive effects of exposure to the environmental contaminant 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) is suppression of the immune system during influenza virus infection. To determine mechanisms by which exposure to

TCDD impairs cell-mediated immunity, we are using a murine model of infection with human influenza A virus. Cell-mediated immunity to influenza virus depends on the activation, proliferation and differentiation of T cells in the mediastinal lymph nodes (MLN). Specifically, activated CD8+ T cells differentiate into cytotoxic T lymphocytes (CTL), which migrate to the lung and kill virus-infected cells. We have previously shown that exposure to TCDD suppresses the generation of CTL in influenza virus-infected mice and attribute this to impaired activation of influenza virus-specific T cells. Thus, we tested the hypothesis that exposure to TCDD impairs the generation of CTL by suppressing proliferation and increasing apoptosis of influenza virus-specific T cells. To follow influenza virus-specific T cells, we used either MHC class I-restricted tetramers or clonotypic antibodies specific for the T cell receptor of influenza virus-specific T cells. For these studies, mice were gavaged with TCDD (10μg/kg) or vehicle one day prior to i.n. infection with influenza virus. Bromodeoxyuridine was added to the drinking water (0.8 mg/ml) to examine proliferation. Mice were killed 3 to 9 days post infection and MLN cells were stained and analyzed using flow cytometry. Exposure to TCDD caused a three-fold reduction in influenza virus-specific CD8+ T cells that were BrdU+. Contrary to our hypothesis, TCDD had no effect on apoptosis of influenza virus-specific CD8+ T cells, based on staining with 7-AAD and annexin V. These findings suggest that decreased cellularity is due to impaired T cell activation, not enhanced cell death. Future experiments will determine whether decreased proliferation is due to the induction of anergy in influenza virus-specific T cells.

867 EXPOSURE OF MICE TO TCDD DECREASES MUCOSAL IMMUNITY AND INCREASES SUSCEPTIBILITY TO ORAL INFECTIONS BY *SALMONELLA TYPHIMURIUM*.

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Study results from several laboratories have shown that 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent immunosuppressive compound. Although antibody production is affected, the cellular immune system is the most sensitive to the immunotoxic effects of TCDD. It is clear that the systemic immune organs are affected by TCDD; however, the effects of TCDD on the mucosal immune system have not been well studied. The studies described in this abstract were designed to define the effects of TCDD on mucosal immune responses to the pathogenic enteric bacterium *Salmonella typhimurium*, and to relate these changes to changes in susceptibility of mice to an oral infection with this organism. It was found that treatment of C57Bl/6 mice with an oral TCDD dose of 15 ug/kg resulted in a significant increase in susceptibility to an oral infection as measured by bacterial numbers in the liver, as well as liver damage determined with histologic procedures and serum levels of ALT. TCDD treatment also was found to inhibit the proliferation of mesenteric lymph node lymphocytes to bacterial antigen. To further define the effects of TCDD, mice treated as above were infected with an attenuated strain of *S. typhimurium*that expresses tetanus toxoid (strain BRD 847). The TCDD-treated mice showed a more severe liver infection and pathologic effects by the attenuated bacteria, as well as suppression of lymphocyte proliferative responses to the bacterial antigen or tetanus toxoid. Serum levels of antibody to tetanus toxoid of all IgG isotypes were less in the TCDD-treated mice, but the mucosal IgA response was not affected by TCDD treatment. These data show that TCDD suppresses mucosal cellular-immune responses and T-cell-dependent antibody production to pathogenic and attenuated *S. typhimurium*.

868 SUPPRESSIVE EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) ON TH2-DERIVED CYTOKINE PRODUCTION IN PRIMARY IMMUNE REACTION OF MICE.

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TCDD has been reported to suppress antigen-specific antibody production, but the precise mechanism remains to be clarified. The type 2 helper T (Th2) cell-derived cytokines are known to be involved in the humoral immune response. We have previously demonstrated that TCDD affects Th2-derived cytokine production by splenocytes in the secondary immune response. In the present study, we investigated whether TCDD affects the Th2-derived cytokine production in the primary immune reaction, where these cytokines play a pivotal role in the proliferation of antigen-specific B cells and their differentiation into antibody-producing plasma cells. Female C57BL/6 mice (6 week-old) were orally administrated TCDD (20 μg/kg) or vehicle alone (corn oil-4% nonane), and simultaneously immunized with ovalbumin (OVA, 100 μg/mouse)/alum intraperitoneally. Four days after the treatment, TCDD significantly suppressed the IL-4, IL-5 and IL-6 production by splenocytes re-stimulated *ex vivo* with OVA, while it did not alter the cell numbers and populations of T and B cells. The production of IL-5 and IL-6, but not of IL-4, was suppressed by TCDD from the day 4 through day 14. Among them, IL-5 was particularly sensitive to TCDD and significantly suppressed at a low dose of TCDD-exposure (1 μg/kg). To clarify whether T cells or antigen-presenting cells (APCs) are attributable to the suppression of cytokine production by TCDD, T cells and APCs were separated from TCDD- and vehicle-treated mice, and subjected to splenocyte-reconstitution cultures. We found that TCDD-treated T cells were essential for the suppression of IL-5 production. In conclusion, these results showed that TCDD exposure suppresses the Th2-derived cytokine production in the primary immune response by mainly affecting T cells, and suggested that these effects of TCDD are involved in the impairment of antigen-specific antibody production.

869 THE EFFECTS OF TCDD ON GERMINAL CENTER FORMATION IN THE SPLEEN OF C57BL/6 MICE.

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2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is known to suppress the antibody production in humoral immune response. However, the mechanisms by which TCDD causes immunosuppressive effects remain to be defined. After thymus-dependent antigen (Ag) immunization, Ag-activated B cells migrate into lymphoid follicles to form germinal centers (GCs). In GCs, B cells undergo proliferation and selection to differentiate into plasma cells that produce high-affinity antibodies. The aim of this study was to elucidate at which stage TCDD affects B cell development. We therefore investigated the effects of TCDD on GC formation. Female C57Bl/6 mice were orally administered 0 (vehicle) or 20 μg/kg of TCDD and immunized intraperitoneally with 100 μg of alum-precipitated ovalbumin (OVA) (day 0). *in ova*lalum system, OVA-specific IgG1 in plasma was detected from day 10 by ELISA and the increased proportion of GC B cells in spleen was detected from day 7 by flow cytometry, so that mice were sacrificed on days 7, 10 and 14 to determine the time course effects of TCDD. Suppression of antibody production by TCDD treatment was observed on days 10 and 14. TCDD significantly reduced cell number of GC B cells, as defined by B220$^+$Peanut agglutinin (PNA)$^+$, from day 7 through day 14, indicating that TCDD inhibited GC formation from its early stage. Besides, the suppression of GC formation by TCDD was also histochemically detected by PNA staining. TCDD did not affect immunohistochemical staining pattern of follicular dendritic cells, which are involved in GC formation. These results suggest that TCDD suppresses antibody production through preventing B cell proliferation or differentiation in GCs.

870 EARLY T CELL CYCLE BLOCK BY HYDROQUINONE AND CATECHOL.

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Cigarette smoke induces profound suppression of T cell responses in the lungs. We have reported that the two major phenolic components of cigarette tar, hydroquinone (HQ) and catechol, block DNA synthesis in proliferating lymphocytes by reversibly inhibiting ribonucleotide reductase. However, inhibition of DNA synthesis following HQ- and catechol-exposure of resting lymphocytes was not reversible, implicating additional mechanisms of inhibition. The purpose of these experiments was to investigate the molecular basis of HQ- and catechol-induced cell cycle arrest. Peripheral blood mononuclear cells (PBMC) were activated with anti-CD3 and PMA in the presence or absence of 50 μM HQ or 50 μM catechol, and a set of key events that mediate progression through G_0- and G_1-phases were analyzed. HQ and catechol blocked cells in the G_0/G_1-phase and also inhibited blast transformation. However, induction of CD25 and CD69 on the cell surface was unaffected, and the production of IL-2 was reduced by only 35-40%, suggesting that HQ- and catechol-treated T cells initiate the activation process, but inhibition of one or more pathways halts further progress. RNA synthesis, which is characteristic of G_1-phase entry and progression, was inhibited by greater than 75% in HQ- and catechol-treated cells. Additionally, HQ and catechol diminished the number of transferrin receptors (TfR) per cell, and western blot analyses revealed that E2F-dependent gene expression, including genes encoding Cdk1 and Mcm2, was reduced by 60-80%. Finally, HQ or catechol exposure prevented dissociation of E2F4 from p130, which may explain reduced E2F activity. The relationship between inhibition of E2F activity and TfR expression is not yet known, but the combined effects clearly contribute to the failure of activated T cells to progress through G_1-phase of the cell cycle. To our knowledge, these studies provide the first explanation of how these benzene derivatives affect the first stage of proliferation in any cell type. *Supported by the Cancer League of Colorado and NIEHS grant ES05673.*

871 EVIDENCE FOR THE INDUCTION OF APOPTOSIS IN IMMUNE CELLS BY DELTA-9-TETRAHYDROCANNABINOL.

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Delta 9-tetrahydrocannabinol (THC), the main psychoactive component of marijuana has been shown to suppress the immune response. However the exact mechanism of THC-induced immunosuppression remains unclear. In the current study we tested the hypothesis that exposure to THC leads to the induction of apoptosis in lymphocyte populations. Splenocytes cultured in the presence of 10 μM or greater concentrations of THC showed significantly reduced proliferative responses to mitogens, including anti-CD3 mAbs, Con A, and LPS *in vitro*. Thymocytes as well as naïve and activated splenocytes exposed to 10 μM or 20μM THC showed significantly increased levels of apoptosis. *in vivo* studies revealed that 10 mg/kg of THC injected i.p. led to thymic and splenic atrophy as early as 6 hours after treatment. THC exposure led to reductions in all subpopulation of splenocytes and thymocytes examined. Functional studies revealed that splenocytes from THC-treated mice had significantly reduced proliferative responses to anti-CD3 mAbs, Con A, and LPS *in vitro*. Finally, examination of apoptosis in the thymus and spleen revealed that exposure to THC resulted in increased levels of apoptotic cells in both thymic and splenic cultures. Together these results suggest that exposure to THC can lead to significant suppression of the immune response by induction of apoptosis. (Supported in part by NIH grants: RO1ES09098 and RO1HL58641).

872 EFFECTS OF ETHANOL ON CYTOKINE INDUCTION BY POLYINOSINIC POLYCYTIDYLIC ACID (POLY I:C).

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Ethanol suppresses Natural Killer (NK) cell activation by poly I:C in a binge drinking model in mice. This leads to diminished resistance to B16F10 tumor cells. Possible mechanisms for this effect are under investigation. In the present study, the effect of ethanol (EtOH) on the induction of cytokines that are important in activation of NK cells was investigated. Mice were treated with poly I:C (0.1 mg/mouse, ip) and EtOH (6 g/kg as a 32% solution in water, by gavage) or corticosterone (18 mg/kg in 2% beta-cyclodextrin, sc) at the same time. Mice were then bled and the spleen was obtained for analysis. RNAse protection assays were used to examine expression of cytokine mRNAs in the spleen 2 hr after dosing (using a custom probe kit from Pharmingen). Ethanol suppressed expression of IFN-alpha, IFN-beta, IFN-gamma, IL-12, and IL-15. Expression of IL-6 was not affected and expression of IL-10 was increased. Because EtOH induces a stress response, corticosterone was evaluated using a dosing scheme that produces the same area under the corticosterone concentration *vs.* time curve as measured in EtOH-treated mice. Corticosterone decreased expression of IFN-alpha, IL-6, and IL-12 but had no effect on the other cytokines. To confirm that changes in mRNA changed the concentration of cytokine proteins, IFN-alpha, IL-12, and IL-10 were evaluated by ELISA (using kits from PBL Biomedical and Pharmingen). All three of these cytokines reached peak levels 4-8 hr after poly I:C. EtOH and corticosterone decreased serum IFN-alpha from ~300 pg/ml to less than 50 pg/ml at 2 and 4 hr. In contrast, EtOH increased IL-10 concentration, whereas corticosterone had no effect. The levels of IL-12 were significantly decreased from ~38, 000 pg/ml to less than 5000 pg/ml at 2 hr. These results indicate suppression of NK cell activating cytokines and enhancement of immunosuppressive IL-10 may contribute to the deleterious effect of EtOH on resistance to tumor cells and that corticosterone is not the major mediator of most of these changes. This work was supported by NIAAA grant AA09505.

873 GALLIUM ARSENIDE INDUCES MACROPHAGE ACTIVATION.

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Gallium arsenide (GaAs), a semiconductor utilized in the electronics and computer industries, has the paradoxical effects of causing systemic immunosuppression and local inflammatory reaction at the exposure site. Macrophages at the exposures site appear activated. To distinguish between GaAs' primary mode of action and secondary consequences, macrophages isolated from unexposed mice and macrophage cell lines were exposed to GaAs *in vitro*. In culture, GaAs upregulated expression of invariant chain, and the proteases, cathepsins B and L, regardless of the cell source. Invariant chain cleavage was also enhanced. Hence, GaAs directly influences macrophages. However, not all phenotypic changes exhibited by macrophages chemically exposed *in vivo* were observed after *in vitro* exposure, such as increased major histocompatibility complex class II expression. These findings raise the possibility that the complete impact of GaAs on macrophages may require cytokine secretion. To study the role of cytokines in GaAs' immunomodulation, DNA microarray analysis and mice having disrupted cytokine genes are being used. The ability of GaAs to activate macrophages may contribute to the chemical-induced inflammation at the exposure site. This work was supported by NIEHS grant ES07199, and M. T. Harrison was supported, in part, by training grant T32 ES07087.

874 CISPLATIN INDUCED GENE EXPRESSION AND ALTERED MORPHOLOGY IN RAT AND HUMAN KIDNEY SLICES.

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To improve the prediction for man, gene expression technology in combination with measurements of cell function and morphology has been applied to rat and human kidney slices and to a rat *in vivo* study, exposed to the broadly used anticancer agent cisplatin. Kidney of rats administered 1 mg/kg for 5 days or a single dose of 8 mg/kg exhibited tubular degenerative and regenerative changes, granular and proteinaceous casts and tubular/intratubular mineralisation at 96 hours. Kidney slices of rat (10-80 μM) and human (40-500 μM) revealed changes consistent with acute nephrosis of the tubular epithelium. In the rat the cellular injury was localised preferentially in the medulla and at the cortico-medullary junction, whereas in the human kidney slices there was no preferred location. Convoluted regions of the tubules were more susceptible to injury followed by the straight and collecting ductules. Immunohistochemistry staining for apoptosis correlated with the location of damage. In both species, the glomerulus appeared resistant even at high concentrations of cisplatin. The gene categories induced by cisplatin were time and dose dependent and preceded a change in morphology in both species. Gene categories included: apoptosis, DNA damage, cell stress, protein damage, cell cycle/proliferation, metabolism and transport-related genes. In summary, cisplatin-induced nephrotoxicity was demonstrated in rat and human kidney slices by changes in gene expression profiles followed by morphological features. Refinement of the understanding of the gene pathways to better define the mechanisms of toxicity and relate them to interspecies differences in morphology is ongoing.

875 CISPLATIN-INDUCED RENAL CELL APOPTOSIS IS p53 AND CASPASE-DEPENDENT AND INDEPENDENT.

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The chemotherapeutic cisplatin causes renal dysfunction and renal proximal tubular cell (RPTC) apoptosis by mechanisms known to involve the activation of caspase 3. The goal of these studies was to determine the mechanism of cisplatin-induced caspase 3 activation and apoptosis. Cisplatin (50 μM) produced time-dependent apoptosis over 24 hr in primary cultures of RPTC, causing cell shrinkage, a 50-fold increase in caspase 3 activity, a 4-fold increase in phosphatidylserine externalization, and 5- and 15- fold increases in chromatin condensation and DNA hypoploidy, respectively. Caspase 8 and 9 activities did not increase at any time during the 24 hr cisplatin exposure, suggesting these enzymes did not mediate cisplatin-induced caspase 3 activation. Immnocytochemistry and immunoblot analysis revealed that cisplatin treatment of RPTC increased nuclear p53 expression as early as 4 hr after treatment. This increase preceded caspase 3 activation and chromatin condensation by 4 hr. Pretreatment with the p53 inhibitor α (2-(2-imino-4, 5, 6, 7-tetrahydrobenzothiazol-3-yl)-1-*p*-tolylethanone) (PFTα, 10 μM) prior to cisplatin treatment inhibited p53 nuclear expression at 4, 8 and 12 hr and totally inhibited phosphatidylserine externalization and caspase 3 activation at 12 hr. In contrast, PFTα only partially inhibited caspase 3 activation, chromatin condensation and DNA hypoploidy after 24 hrs of exposure. Neither the caspase 3 inhibitor DEVD-fmk nor the general pan caspase inhibitor ZVAD-fmk (50 μM) inhibited cisplatin-induced p53 nuclear expression. Both DEVD-fmk and ZVAD-fmk completely inhibited caspase-3 activity but, like PFTα, only partially inhibited cisplatin-induced chromatin condensation, annexin V labeling and DNA hypoploidy after 24 hr. These data demonstrate that approximately 50% of cisplatin-induced apoptosis is dependent on p53 and caspase 3 while another 50% occurs independently of both proteins.

876 DISRUPTION OF N- AND KSP-CADHERIN LOCALIZATION AND EXPRESSION IS ASSOCIATED WITH MERCURY-INDUCED ACUTE RENAL FAILURE.

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Adhesion molecules play a critical role in maintaining the integrity and polarity of proximal tubular epithelial cells. Although a disruption of cell-cell interactions is suggested in acute renal failure (ARF), little information is known about the expression or integrity of both zonula occludens (tight junctions) and zonula adherens (cadherin) during ARF. The purpose of this study was to investigate the expression and localization of adhesion molecules during mercury chloride-induced

ARF. Male C3H mice were challenged with 25 μM/kg mercury i.p. Plasma creatinine and blood urea nitrogen were significantly elevated at 12, 24, and 48 hr, indicating a reduction of renal function. Western blot analysis revealed no major changes in the expression of tight junction components, ZO-1, ZO-2, or claudin-1 at 48 hr. In addition, no change in the expression of E- or K-cadherin, or β-catenin was seen. However, large decreases in N- and Ksp-cadherin, and α- and p120 catenin were observed. These decreases were not observed at 6 and 12 hr, but were seen following 24 hr of mercury challenge. Immunohistochemistry demonstrated that the Ksp-cadherin was localized along the lumen of renal proximal and distal tubule. At 48 hr, there was a distinct loss of immunoreactivity, and a loss of luminal localization. Similar findings were seen with N-cadherin. Our results indicated that Hg-induced nephrotoxicity is associated with distinct changes in the distribution and expression of zonula adherens proteins, most notably Ksp- and N-cadherin, in the absence of alterations in the expression of zonula occludens consituents. These results further our understanding of toxicant-induced renal failure, and the potential role of adhesion molecules as molecular targets of xenobiotics.

877 SELECTIVE LOSS OF N-CADHERIN EXPRESSION IN THE KIDNEYS OF AGED FISHER 344 RATS, BUT NOT SHR RATS.

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Aging is associated with a loss of renal reserve, and increased sensitivity to either xenobiotic or physiologic insult. Given the critical role of adhesion molecules in maintaining the integrity and polarity of proximal tubular epithelial cells, it was hypothesized that loss of zonula occludens (tight junctions), zonula adherens (cadherin), or desmosomal proteins may be associated with aging. The purpose of this study was to investigate the expression and localization of adhesion molecules in young (3-4 month) and old (24-26 month) male Fisher 344 rats. Western blot analysis revealed no difference in the expression of several components of tight junctions, including occludin, claudin-1, ZO-1 or ZO-2. Similarly, no differences in the expression of desmocollin, desmoglein, or desmoplakin, constituents of desmosomal junctions, were observed. However, a pronounced loss of N-cadherin was observed, in the absence of major changes in the expression of E- or Ksp-cadherin. The decreased protein expression of N-cadherin cannot be accounted for by solely by the loss of proximal tubular cells since Ksp-cadherin is expressed in an overlapping population of cells. As N-cadherin is also present in neurons projecting to the kidney, immunohistochemical analysis was used to demonstrate that the loss of N-cadherin was localized to proximal tubular epithelium. Decreased protein expression of two catenins, α- and β- was also observed in the aged kidneys with only slight changes seen with γ- or p120 catenin expression. Taken together, these data suggest that aging is associated with decreased expression of N-cadherin and α- or β-catenin, in the absence of significant changes in other adhesion molecules.

878 ADVANCING AGE AND GLOMERULAR LOCATION PLAY SIGNIFICANT ROLES IN THE DEVELOPMENT OF RENAL DISEASE IN LABORATORY DOGS.

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It is well documented that the incidence of renal disease, and therefore renal dysfunction, increases with age in many species of mammals. Such alterations in renal structure and function may significantly affect long-term toxicology studies. The purpose of this study was to assess the temporal evolution of glomerulosclerosis, an important renal lesion, in laboratory housed dogs, an important model system in chronic toxicology studies. We histopathologically examined representative sections of dog kidneys, quantified glomerular lesions (using the 0-5 scale of the World Health Organization classification system) and performed of statistical analysis of extent and distribution of such changes. The kidney samples were obtained by necropsy, and occasionally biopsy, procedures from a collection of 159 purebred Beagle dogs maintained for their entire lifespan in well-controlled conditions. The lesions were correlated with sex, age, and intra-renal location of affected glomeruli to determine the relationship of each in the development of glomerulosclerosis. All dogs examined had some degree of glomerulosclerosis. In the youngest (up to 2 years of age), this was minimal, but was more advanced by middle age (3-7 years). The condition progressed with further aging and was associated with progressive fibrosis and tubular loss. Location and advancing age were significantly related to the development of glomerulosclerosis such that as age increases, the incidence of glomerulosclerosis increases, with the inner ray and inner cortex demonstrating the highest occurrence ($p<0.0001$ for both). This study indicates that glomerulosclerosis is progressive over the lifetime in a genetically similar population of laboratory Beagle dogs maintained under optimal standard environmental conditions. This information needs to be taken into consideration when conducting chronic toxicological experiments using such animals.

879 MERCURY DECREASES TRANSEPITHELIAL RESISTANCE IN MDCK I CELLS: ASSOCIATION WITH ALTERATIONS IN CADHERIN/CATENIN COMPLEXES.

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The loss of transepithelial resistance (TER) in confluent MDCK I monolayers has been used extensively as an *in vitro* model to investigate mechanisms associated with disruption of cell-cell adhesion. The current studies were designed to determine if mercuric chloride is associated with a reduction of TER in MDCK I cells. Cells were seeded on insert 12-well Transwell® insert chambers and following 8 days in culture the cells were challenged with mercuric chloride (0-25 μM) in HBSS. A dramatic reduction in TER was seen with 25 μM mercury at all timepoints examined (15-120 min). A loss of TER was also seen with 12.5 μM mercury at 60 and 120 min, with no effect seen at 6.25 μM. The finding that a more dramatic reduction in TER was seen at 15 min when 25 μM mercury was added to the basolateral compartment *versus* the apical chamber (29 & 52.5% control, respectively) suggests that the adherens junction (cadherin/catenin complex) may be disrupted prior to changes in the zonula occludens (tight junction). This finding is supported by Western blot data that demonstrate a small, but significant, increase in the amount of β- and p120 catenin in the Triton X-100 soluble fraction of MDCK cells at 15 and 60 min following mercury challenge. These data suggest that *in vitro* mercury challenge is associated with a disruption of cell-cell adhesion, as evidenced by the loss of TER, and suggest that alterations in cadherin/catenin complexes may precede changes in the tight junction following mercury challenge.

880 TISSUE REPAIR: A KEY TO SURVIVAL FOLLOWING NEPHROTOXIC CHALLENGE.

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Trichloroethylene (TCE), a common industrial degreasing solvent, is produced in the United States at about 130, 000 metric tons per year. One of the primary mechanisms hypothesized for TCE-induced renal damage and tumors in humans and rodents is *via* the glutathione-dependent β-lyase-mediated formation of S-(1, 2-dichlorovinyl)-L-cysteine (DCVC) metabolite *in vivo*. Our preliminary studies suggested that a timely and adequate renal tissue repair response results in survival of the animals from a low or medium toxic dose of DCVC. However, inhibition of tissue repair, known to occur after a high dose of DCVC, leads to animal death by renal failure. The objective of this study was to investigate the effects of modulation of tissue repair on the ultimate outcome of renal toxicity. When cell division and timely tissue repair were blocked with colchicine, an ordinarily non-lethal dose of DCVC (30 mg/kg, ip) resulted in 90 % mortality suggesting that patency of cell division is very critical for recovery from renal injury of DCVC. The next strategy was to "preplace" renal tissue repair by two different approaches. First approach was to administer a low dose (15 mg/DCVC/kg, ip) 72 h prior to administering a normally lethal dose (75 mg/kg, ip). Second approach was to administer mercuric chloride (6 mg/kg, ip), a well-known nephrotoxicant, 96 h prior to a lethal dose of DCVC. In both the cases augmented and sustained cell division and tissue repair (^{3}H-thymidine pulse labeling and PCNA immunohistochemistry) induced by the priming low dose resulted in 100 % survival inspite of initial massive renal injury. These findings clearly suggest that tissue repair is the key mechanism for survival following an ordinarily lethal nephrotoxic challenge. These results point to novel post-exposure pharmacotherapeutic avenues for treatment of acute renal failure to change the clinical course from hopeless to one of a positive prognosis.

881 CALPAINS MEDIATE PROTEOLYSIS OF PAXILLIN, VINCULIN, AND TALIN DURING ACUTE RENAL CELL DEATH.

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Calpains, Ca^{2+}-activated neutral cysteine proteases, play a critical role in acute renal cell injury/death. However, their cellular substrates in this process remain poorly defined. Paxillin, vinculin, talin, and α-actinin are cytoskeleton-associated proteins that have been reported to be calpain substrates. The goals of the present study were to determine 1) if paxillin, vinculin, talin and α-actinin are calpain substrates during acute renal cell death, and 2) the effects of the calpain inhibitors PD150606 (a

Ca^{2+}-binding site inhibitor) or SJA7029 (a catalytic site inhibitor) on these proteins during cell death. Rabbit renal proximal tubules (RPT) were exposed to antimycin A (10 μM) or subjected to hypoxia for 30 min. LDH release was used as a marker of cell death. Using immunoblot analysis, rabbit RPT expressed 68-kDa paxillin, 117-kDa vinculin, 210-kDa talin, and 100-kDa α-actinin. Treatment of control RPT lysate with purified μ-calpain resulted in decreased parental forms of paxillin, vinculin, talin, but not α-actinin. These results suggest that paxillin, vinculin, and talin, but not α-actinin, are *in vitro* calpain substrates. Antimycin A exposure and hypoxia decreased cytoskeleton-associated paxillin, vinculin, and talin levels, altered RPT morphology, and increased LDH release. α-Actinin levels were not altered during antimycin A- or hypoxia exposure. PD150606 (100 μM) or SJA7029 (100 μM) preserved cytoskeleton-associated paxillin, vinculin, and talin levels, preserved RPT morphology, and blocked LDH release. The results demonstrate that calpains cleave paxillin, vinculin, and talin during acute renal cell death, and suggest that calpain proteolysis of these substrates may lead to the cell death morphology.

882 QUANTITATION OF THE DOSE-RESPONSE FOR FORMATION OF DNA ADDUCTS IN RAT LIVER BY 2-ACETYLAMINOFLUORENE.

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We have developed an initiation/promotion model of rat liver carcinogenesis to quantify initiation effects of genotoxic carcinogens at low exposures for 12 weeks followed by phenobarbital promotion for 24 weeks to elicit tumor development. In previous studies, we demonstrated non-linearities and no effect levels for some of the critical early events of initiation, including DNA adduct formation, inhibition of bioactivation, liver cell toxicity and compensatory hepatocellular proliferation and induction of preneoplastic lesions, as well as no-effect levels for promotable neoplasia, for 2-acetylaminofluorene (AAF) (*Toxicol. Sciences.*, **45**, 152, 1998) and diethylnitrosamine (*Arch. Toxicol.*, **73**, 394, 1999). With AAF at the lowest cumulative exposure (126 μmol/kg bw over 12 weeks), DNA adducts were formed, but no promotable liver neoplasia was found. At the next highest exposure (420 μmol/kg bw), DNA adducts were about 4-fold higher and weak promotable neoplasia (1 adenoma) was observed. The purpose of the present study was to assess DNA adduct formation and initiation at similar and lower exposures than those used in the previous study. As before, AAF was administered over 12 weeks for cumulative exposures of 4.2, 42, 420 (the mid dose from the previous study), or 840 μmol/kg/bw followed by 4 weeks recovery. Increases occurred in relative liver weights, but no increase in plasma alkaline phosphatase or transaminases was evident. DNA adducts, expressed as % of the highest dose (100%) were 0.8, 2.9 and 21.8% for the other exposures, respectively, and 1.1% for the controls. The controls showed 1 spots, one of which was co-chromatographic with the AAF-DNA adducts thus limiting detection of the latter at the lowest dose. Further efforts are underway to resolve this analysis. We conclude that AAF forms DNA adducts at exposures below those producing even early biological markers of initiation.

883 ACCELERATOR MASS SPECTROMETRY: A TOOL FOR TRACING ENVIRONMENTALLY-RELEVANT DOSES OF ^{3}H AND ^{14}C-LABELED CARCINOGENS.

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Accelerator mass spectrometry (AMS) is a nuclear physics technique for quantifying rare, long-lived isotopes with high sensitivity and precision. Consequently, AMS has facilitated *in vivo* studies at environmentally relevant exposure levels of carbon-14 and tritium labeled carcinogens. We have performed low-level dosimetry studies in laboratory animals, hence establishing the dose-responses for covalent binding of compounds to DNA and protein at ng/kg body-weight doses, with DNA adduct level detection limits as low as 1 adduct/10^{11}nucleotides. More recently, human studies have been conducted in which volunteers have been administered dietary relevant doses of heterocyclic amine food mutagens. Adduct levels were 10-fold higher in humans than in rats administered the equivalent dose. Through these studies we are able to more accurately determine the risk associated with environmental exposure to carcinogens. The work was conducted under the auspices of the US DOE by LLNL (W-7405-ENG-48) and partially supported by NIH (NCRR) and DOD.

884 DNA ADDUCT CONFORMATION DEPENDS ON DNA CONFORMATION.

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Benzo[*a*]pyrene is metabolically activated to the highly mutagenic derivative (+)-7*R*, 8*S*-dihydroxy-9*S*, 10*R*-epoxy-7, 8, 9, 10-tetrahydrobenzo[*a*]pyrene (BPDE). BPDE-adducts can be one of two stereoisomers and each can adopt several conformations. BPDE-adducts along the DNA are repaired at different rates suggesting that sequence context influences repair rate. How sequence context alters repair efficiency is unknown. Our hypothesis is that adduct conformation plays a key role in the recognition and incision of adducts by the NER system and that DNA conformation influences stereo-specific structure and conformation of BPDE-adducts. We examined the role of DNA conformation in the formation of BPDE-adducts and in the recognition and excision of BPDE-adducts by *B. caldotenax* UvrABC. Both supercoiled and linear plasmid DNAs were reacted with BPDE and subjected to uvrABC incision. Incision efficiency on adducts formed in supercoiled DNAs were 2-5 fold greater than of adducts formed in linear DNAs. Linearizing supercoiled plasmid DNAs after BPDE adduct formation did not diminish incision efficiency. Heat denaturation and reannealing of supercoiled plasmids decreased incision efficiency suggesting that the conformation of the adducts changed. Others have shown that *E. coli* UvrABC more efficiently incises (+)-*cis*-BPDE isomers than (+)-*trans*-BPDE isomers which were both site-specifically constructed into 50-mer substrates. If *B. caldotenax* UvrABC works similarly to *E. coli* UvrABC, our results suggest that on supercoiled DNA (+)-*cis*-BPDE isomer adducts are predominantly formed, whereas on linear DNA the (+)-*trans*-BPDE isomer adducts predominate. These results support our hypothesis, and these stereoisomeric lesions can serve as a conformational structural tool not only for exploring the mechanism of DNA damage processing by NER but also to provide insights into the potential impact of lesions on mutagenesis. (Supported by NIH Grant 1R01-ES06460 and the Commonwealth of Kentucky Research Challenge Trust Fund.)

885 IDENTIFICATION AND CHARACTERIZATION OF THREE NOVEL N^2-DEOXYGUANOSINE ADDUCTS OF DIEPOXYBUTANE.

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1, 3-Butadiene (BD) is a known rodent and human carcinogen, but the molecular mechanisms of its carcinogenicity are not well understood. Diepoxybutane (DEB), a potent mutagenic metabolite of BD, has been suggested to play a significant role in BD-induced carcinogenicity, and has been reported to react with 2'-deoxyguanosine (dG) to yield *N*-7-(trihydroxybutyl)guanine adducts. In the present study, three novel dG adducts of DEB were characterized from the reaction of DEB and dG at physiological conditions (pH 7.4, 37°C). The adducts were purified from the reaction mixture using Sephadex-LH-20 chromatography and semi-preparative C-18 HPLC. The structures of the adducts, as determined by ESI MS and NMR (including ^{1}H, ^{1}H-COSY, ^{13}C, ^{13}C DEPT135 and HMQC spectra), were N^2-(2-hydroxy-3, 4-epoxy-1-yl) deoxyguanosine and a diastereomeric pair of N^2-(1-hydroxy-3, 4-epoxy-2-yl) deoxyguanosine. Time-course experiments under different pH conditions indicated that the three adducts were formed readily under both neutral and alkaline conditions (pH 10.0) and that the isolated products were stable for several days under purification and storage conditions. A degradation product of N^2-(2-hydroxy-3, 4-epoxy-1-yl)deoxyguanosine was obtained under certain lyophilization conditions and characterized as N^2-(2-hydroxy-3, 4-epoxy-1-yl)guanine by MS and NMR. These results, which shed new light on the reactivity of DEB, may aid in the development of useful biomarkers for DEB. The results may also contribute to a better understanding of the molecular mechanisms of BD- and DEB-induced mutagenicity and carcinogenicity (This research was supported by NIH Grant ES06841 from the National Institute of Environmental Health Sciences).

886 SYNTHESIS, CHARACTERIZATION AND *IN VITRO* IDENTIFICATION OF N^7-GUANINE ADDUCT OF 2-BROMOPROPANE.

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Recently, we have reported that 2-bromopropane might have an immunotoxic potential in rats when exposed for 28 days. In the present studies, the possibility of 2_i^--deoxyguanosine adduct formation by 2-bromopropane was investigated *in vitro* to elucidate molecular mechanism of 2-bromopropane-induced immunosuppression. N^7-Guanine adduct of 2-bromopropane (i.e., N^7-isopropyl guanine) and its derivatives were chemically synthesized and structurally characterized by analysis of UV, ^{1}H NMR, ^{13}C NMR, COSY and fast atom bombardment mass spectrometry to use as a reference material. Incubation of 2_i^--deoxyguanosine with an excess amount of 2-bromopropane in PBS buffer solution, pH 7.4, at 37°C for 16 hr, followed by a thermal hydrolysis, produced a detectable amount of N^7-isopropyl guanine by an HPLC analysis. In addition, calf thymus DNA was incubated with an excess amount of 2-bromopropane in PBS buffer solution, pH 7.4, at 37°C for 16

hr. After a thermal hydrolysis, followed by removal of DNA by a microfiltration, N^7-isopropyl guanine was detedcted by an HPLC analysis. The present results suggest that 2-bromopropane might form a DNA adduct in N^7 position of 2_i^--deoxyguanosine at a physiological condition.

887 *IN VIVO* AND *IN VITRO* ADDUCT FORMATION AND MUTATIONAL SPECTRA OF S-(2-CHLOROETHYL)-GLUTATHIONE AND N-ETHYL-N-NITROSOUREA IN THE P53 GENE.

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Mutations on p53 tumor suppressor are common in diverse types of human cancers. It is known that p53 binds to DNA in a sequence-specific manner and activates several genes that induce cell cycle arrest or apoptosis following DNA damage. Most of the mutated p53 genes found in human tumors are deficient in sequence-specific trans-activation. The central domain of p53 (encoded by the exons 5 to 8) includes the residues responsible for binding to specific DNA sequences. We employed a yeast functional assay consisting of a double-selection method (Environment Mol. Mutagenesis 35:31-8, 2000) to characterize the nature of N-ethyl-N-nitrosourea (ENU) and S-(2-chloroethyl)-glutathione (CLEGS)-induced mutations of p53 trans-activating activity, in a physiological environment. Both mutagens produced transitions, mostly on G-rich regions. However, the sequence analysis revealed different patterns of hotspots depending on the mutagen used. With ENU high frequency of mutation was observed at codons 141, 177, 194, 199, and 278; with CLEGS high frequency was observed at codons 152, 177, 244, and 279. On the other hand, a ligation mediated polymerase chain reaction (LM-PCR) assay, using fluorescent-labeled primers, was developed to establish if those hotspots corresponded to the sites where adducts are produced *in vivo* and *in vitro* by CLEGS. We observed a complex pattern of adduced nucleotides, preferentially on G-rich sequences along both strands in exons 5 to 8 of p53. This profile of adducts did not correspond to the pattern of hotspots observed, indicating that different factors are responsible for mutation fixation inside the cell than those revealed by the LM-PCR analysis.

888 THE SCREENING OF TARGET CANDIDATE REGIONS SUSCEPTIBLE TO MUTATIONS IN THE GENOME OF HAMSTER KIDNEY TUMORS INDUCED BY STILBENE ESTROGEN.

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Diethylstilbestrol (DES) is a renal carcinogen in Syrian hamsters. However, the mechanism of DES-induced carcinogenesis is not clear. In order to understand the sequential molecular events involved in the DES-induced tumorigenesis, it is important to identify the genomic target candidate regions susceptible to mutations. In this study, we used the Random Amplified Polymorphic DNA (RAPD), a polymerase chain reaction based fingerprinting assay, to screen the entire genome for mutations in DES induced hamster kidney tumors. Tweleve out of hundred primers used for RAPD amplification revealed 22 amplification products harboring mutation(s) in DES induced hamster kidney tumor genome as compared to the age matched untreated control kidney genome. Further characterization revealed that some of these regions harboring mutations are from the coding regions of the kidney tumor genome. Gene expression analysis by RT-PCR revealed that one of the uncharacterized mutated genes is downregulated by several folds in the kidney tumors as compared to the age-matched controls. Findings of this study indicates that RAPD is an efficient method to identify the target candidate regions in the genome that are susceptible to mutations induced by exposure to synthetic estrogen, DES.

889 MECHANISTIC STUDIES OF ACRYLAMIDE INDUCED CARCINOGENICITY IN THE F344 RAT.

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Chronic treatment with acrylamide in drinking water increased the incidences of adrenal pheochromocytomas, mesotheliomas of the tunica of the testes, and follicular adenomas of the thyroid in male F344 rats. The mechanism(s) for these chronic effects of acrylamide is unresolved. The present studies were conducted to determine whether acrylamide or an acrylamide metabolite altered cell growth in the target tissues in the rat. Specifically, DNA synthesis, mitosis and apoptosis were examined in F344 rats treated with acrylamide (0, 2, or 15mg/kg/day) for 7, 14, or 28 days. Acrylamide treatment produced an increase in DNA synthesis in the target tissues (thyroid, testicular mesothelium, adrenal medulla) at all doses and time points examined. In contrast, in a non-target tissue (liver), no increase in DNA synthesis was seen. No changes in apoptosis or mitosis were observed in any of the tissues examined. Blockage of oxidative metabolism of acrylamide using 1-aminobenzotriazole reduced acrylamide-induced DNA synthesis only in the adrenal medulla. Examination of DNA damage using the Comet assay (single cell gel electrophoresis) showed an increase in DNA damage in the target tissues, but not in non-target tissue (liver). In summary acrylamide causes both an increase in DNA synthesis and DNA damage in the previously reported tumor target sites suggesting that these events in concert may contribute to the observed acrylamide-induced carcinogenicity in the rat.

890 INDUCTION OF MICRONUCLEI IN RAT HEPATOCYTES FOLLOWING DNA DOUBLE-STRAND BREAKS CAUSED BY FURAN TOXICITY *IN VITRO*.

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Furan is hepatotoxic and hepatocarcinogenic in rodents but is not mutagenic. Our laboratory has shown that furan metabolites deplete hepatocyte ATP and activate endonucleases that produce DNA double-strand breaks (dsb). We hypothesize that misrepair of DNA dsb results in mutations involved in furan carcinogenesis. Our objective was to determine whether misrepair of furan-induced DNA dsb leads to cytogenetic events such as micronuclei formation. F-344 rat hepatocytes were incubated in suspension with furan (0 to 100 μM) for up to 3 hr and then placed in monolayer culture with EGF (50 ng/ml) for 72 hr. The number of hepatocytes with micronuclei stained with DAPI and the mitotic index were determined by fluorescence microscopy. Flow cytometry analysis of BrdU incorporation was used to evaluate cell cycle effects. The spontaneous micronuclei frequency was 6.0 ± 0.7%. Furan treatment significantly increased the frequency of micronuclei in a concentration- and time-dependent manner but did not affect mitotic indices. The micronuclei frequencies for the 3-hr exposure to furan at concentrations of 2, 10, 30, and 100 μM were 7.0 ± 0.4%, 10.3 ± 2.1%, 13.1 ± 1.7%, and 17.8 ± 1.2%, respectively. Both inhibition of furan biotransformation by the cytochrome P450 inhibitor 1-phenylimidazole (200 μM) and inhibition of the endonuclease activity by aurintricaboxylic acid (100 μM) prevented micronuclei induction, indicating a requirement for metabolic activation and the need for endonuclease-mediated DNA damage. The labeling index of hepatocytes treated with furan at 30 μM for 3 hr (61.0 ± 6.2%) was significantly greater than that of control cultures (17.8 ± 3.6%), indicating the induction of DNA synthesis. The results of these studies show that hepatocyte DNA dsb formed by furan-induced activation of endonucleases lead to cytogenetic events such as micronuclei formation. The cytogenetic events coupled with furan-induced cell proliferation in the livers of treated rodents are likely to be involved in the development of hepatocellular carcinomas.

891 TOXICITY AND CARCINOGENICITY OF BENZO(A)PYRENE IN SKIN OF DNA REPAIR-DEFICIENT MICE.

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In the present study, we hypothesized that transcription coupled repair (TCR) and global genomic repair (GGR) might modulate BENZO(A)PYRENE (B[a]P)-induced toxicity and carcinogenicity, respectively, in the skin of XP knockout (KO) mice belonging to subgroups C (XPC) and A (XPA). For this purpose, we treated wild type (WT), and XPC and XPA KO mice topically with B[a]P as a single (20, 50 or 200 nmol [group#1]) or multiple doses (20, 50, 100 or 200 nmol once-weekly for 3 weeks [group#2] or 200 nmol once-weekly for 6 weeks [group#3]). Levels of B[a]P diol epoxide-deoxyguanosine (BPDE-dGuo) adducts, hyperplasia and bromodeoxyuridine labeling index (LI) were examined in the epidermis at different time points. Preliminary results showed that Group#1 mice did not differ in the level and disappearance of DNA adducts from epidermal DNA after a single dose of B[a]P, except in XPA KO mice, adducts disappeared more rapidly at the highest dose (200 nmol) tested, which paralleled increased epidermal hyperplasia and LI. In group#2 mice, levels of BPDE-dGuo adducts were similar between all backgrounds of mice at 20 nmol dose of B[a]P. However, at and above 50 nmol of B[a]P XPC KO mice had higher adduct levels than both WT and XPA KO mice. Also in group#3 mice, BPDE-dGuo adduct levels in XPA KO mice were lower than WT and XPC KO mice after multiple doses (200 nmol) of B[a]P. Notably, the decrease in adduct levels in XPA KO mice (Groups#2 & #3) again paralleled increased epidermal hyperplasia and LI suggesting that the lower levels of BPDE-dGuo adducts were due to dilution with newly synthesized DNA. Results from a complete carcinogenesis experiment with several doses of B[a]P suggest that the hyperplasia and cell proliferation induced by higher doses of B[a]P in XPA KO mice leads to a promoting stimulus. XPC KO mice appeared to be more sensitive to

B[a]P complete carcinogenesis at low doses compared to WT or XPA KO mice suggesting that GGR may play an important role in modulating carcinogenesis by PAH in mouse skin.(Supported by grants CA16672 & ES07784)

892 ARSENIC COCARCINOGENESIS: A NEW PARADIGM.

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Epidemiological evidence strongly associates arsenic exposure with increased cancer risk, yet attempts to induce carcinomas in animals have generally failed. This has hindered mechanistic studies of arsenic carcinogenesis. Low concentrations of arsenite are not mutagenic but act as comutagens, most likely due to inhibition of DNA repair. There is evidence that both base and nucleotide excision repair are compromised. However, in biochemical assays, no specific repair enzyme has been found to be sensitive to low concentrations of arsenite, leading to the hypothesis that the effects of arsenite on DNA repair may result from faulty DNA damage-inducible signaling which controls DNA repair. In a test of this hypothesis, Vogt & Rossman (2001) recently showed that in cells treated with submicromolar arsenite and ionizing radiation, the p53-dependent increase in p21 expression, normally a block to cell cycle progression after DNA damage, is deficient. In addition, arsenite upregulated cyclin D1. These results suggest that the failure to find an animal model for arsenic carcinogenesis is because arsenite is not a carcinogen per se, but rather acts as an enhancing agent (cocarcinogen) with a genotoxic partner. We tested this hypothesis with solar UVR as partner in hairless Skh1 mice. Mice given 10 mg/l sodium arsenite in drinking water for 26 weeks had a 2.4-fold increase in tumors yield after 1.7 KJ/m2 UVR 3 times weekly compared with mice given UVR alone. The tumors were mostly squamous cell carcinomas, and those occurring in mice given UVR plus arsenite appeared earlier and were much larger and more invasive than in mice given UVR alone. Mice exposed to arsenite showed increased skin proliferation. These results are consistent with the hypothesis that arsenic acts as a cocarcinogen with a second (genotoxic) agent by inhibiting DNA repair and/or enhancing positive growth signaling. Since arsenic exposure is also associated with lung cancer in humans, it is likely that similar effects will be found in lung cancer as well. This work was supported by NIEHS grant ES09252.

893 SPONTANEOUS AND DNA DAMAGE INDUCED HOMOLOGOUS RECOMBINATION IN MICE DEFICIENT FOR Atm OR p53

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DNA damage is a consequence of both environmental exposure and normal metabolism. A cell is therefore constantly reacting to and repairing DNA damage, the p53 damage response pathway being central to this response. For example, following ionizing radiation, ATM is activated, which then activates p53, which transcriptionally upregulates p21/CIP1/WAF1 and GADD45 to coordinate cell cycle and DNA repair. We have demonstrated that all types of DNA damage induce homologous recombination (HR), leading us to ask whether a relationship exists between the p53 damage response pathway and HR repair. The pink-eyed unstable (pun) murine mutation, a 70 kb tandem duplication within the p locus, results in a dilute coat color and a transparent retinal pigment epithelium (RPE). Reversion by HR deletion of the duplication occurs spontaneously in premelanocytes of the embryo, to produce, after clonal expansion, pigmented cells visible in both the fur and RPE. Such deletion events can be induced by DNA damage. This system allows the examination of both timing of spontaneous HR events, the relative frequency of HR and its inducibility by carcinogens. We have examined the pattern of spontaneous HR in mice with mutations in Atm, p53, p21 and Gadd45. Atm deficient mice display an increasing frequency of HR events during embryogenesis. In contrast, p53 and Gadd45 deficient mice display an increased level of HR only early during embryogenesis, but not later. p21 deficient mice do not display an increased frequency of HR events but do demonstrate a developmental defect in RPE patterning. We have gone on to examine the inducibility of HR in the absence of either Atm or p53 using the differently acting genotoxins X-ray, cisplatin and benzo(a)pyrene. Atm appears to be necessary for X-ray induction of HR, whereas p53 appears to be necessary for both X-ray and cisplatin induction of HR. Neither Atm nor p53 appear to be necessary for benzo(a)pyrene induction of HR. Supported by the NIH (RO1 ES 09519-01, RCDA ES00299 and F32-GM-19147)

894 IDENTIFICATION AND FUNCTIONAL STUDY OF POLYMORPHIC HUMAN XPA GENES.

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XPA initiates excision complex formation in nucleotide excision repair (NER). XPA mutations cause NER deficiency and severe xeroderma pigmentosum (XP) disease. XP patients have a 2, 000-fold higher incidence of skin cancer resulting from impaired repair of UV induced photoproducts. We hypothesize that there are polymorphic XPA alleles that do not eliminate UV-photoproduct repair and thus do not cause XP disease. These polymorphic XPA alleles may reduce repair of chemically induced DNA damage and may be associated with increased incidence of chemically induced cancers. The XPA DNA binding domain is encoded by exons 4 and 5. Exon 6 encodes the transcription factor IIH (TFIIH) binding domain. To identify XPA polymorphisms, PCR amplified fragments containing exons 4, 5 or 6 from 180 random pathology specimens were sequenced. High quality sequence was obtained from fragments containing exon 4 from 129 samples, exon 5 from 146 samples, and exon 6 from 103 samples. Four SNPs and one single nucleotide deletion were found in intron 4. No polymorphisms were found in exons 4 and 5. Only one intron 4 SNP (T to G at -154 bp 5'of exon 5) creates an alternative splice site. Three single nucleotide polymorphisms (SNPs) were found in exon 6 all 3 of which cause missense changes (Arg228Leu, Val234Leu, Leu251Val). The Arg228Leu and Val234Leu alleles are being tested for functional effects on nucleotide excision repair capacity. Survival assays suggest that Arg228Leu and Val234Leu have equivalent survival to the wild type allele for both UV survival and benzo[*a*]pyrene-7, 8-dihydrodiol-9, 10-epoxide (BPDE) survival. Gene specific repair in the DHFR gene shows that the polymorphic XPAs have repair capacity for UV photoproducts equivalent to wild type XPA. Gene specific repair of BPDE-induced DNA damage is currently being performed. Supported by NIH grants ES06460 and RR11803 and funds from the University of Louisville, Kentucky EPSCoR, Markey Cancer Center and Commonwealth of Kentucky Research Challenge Fund.

895 CARCINOGENIC STUDIES WITH ARSENICAL SPECIES IN FEMALE TG.AC (ζ-GLOBULIN-PROMOTED V-HA-RAS) TRANSGENIC MICE.

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Studies have shown that arsenic potentiates 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced skin papillomas in the TgAC (ζ-globin promoted v-Ha-ras) transgenic mouse model. To characterize the carcinogenicity of arsenic, female homozygous TgAC mice were administered 4 treatments of 2.5 μg TPA followed by one of the following arsenicals administered in water: an inorganic arsenical, sodium arsenite (NaAs) or sodium arsenate, or a pentavalent methylated arsenical, monomethylarsinic acid (MMA) or dimethylarsinic acid (DMA). NaAs (20, 50, and 100 mg arsenic/L) enhanced the papilloma response in TPA-treated mice as compared to mice treated only with TPA. Sodium arsenate increased the incidence of papillomas at doses of 50 and 200 mg arsenic/L, but not at the 100 mg arsenic/L level. The latency and peak number of papillomas were not increased at any dose of sodium arsenate. MMA (500 and 1500 mg arsenic/L) and DMA (200 and 1000 mg arsenic/L) did not increase papilloma induction. These data indicate that only NaAs enhanced papilloma induction in TPA-treated TgAC mice. To discern the role of oxidative damage in NaAs-potentiated skin papillomas, TPA-treated female homozygous TgAC mice were also treated with Vitamin E acetate (Vit E) or Natural Anti-Oxidant (NAO). NaAs (0.015%) was administered beginning at week 3. Vit E or NAO treatment alone had little effect on papilloma induction. The papilloma response of animals treated with Vit E or NAO in conjunction with NaAs was not significantly altered from the response of animals treated with NaAs alone. These data demonstrate the usefulness of the TgAC model system to evaluate tumor induction in the skin and demonstrate that there are different skin carcinogenic responses among arsenical species. The lack of response to antioxidant treatment suggests that arsenic potentiates skin tumors through a mechanism other than the generation of oxygen radicals. (This work was sponsored by NIEHS [contract no. NO1-ES-95442]).

896 DICHLOROACETIC ACID (DCA) TOXICITY STUDY USING Tg.AC AND p53 TRANSGENIC MICE.

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DCA, a disinfection by-product (DBP) of drinking water chlorination/ozonization, was administered to Tg.AC and p53 transgenic mice to determine whether these transgenic strains could serve as models to identify the carcinogenic potential of DBP chemicals. In addition, the models may serve to rank families of DBP chemicals for toxicity. Tg.AC and/or p53 mice were exposed to DCA in drinking water (0, 500, 1000 or 2000 mg/L) or by topical application (0, 31.25, 125, or 256 mg/Kg/day) for 26 (Core) or *gE* 39 (Special) weeks. In the drinking-water study, Tg.AC and p53 mice exhibited decreased body weight (>10%) and water consumption, increased liver weight (>10%), lung neoplastic changes (alveolar-bronchiolar adenoma; male Tg.AC mice only), and liver non-neoplastic changes. In the dermal study, Tg.AC mice exhibited an increased incidence of papillomas at the

site of application, increased liver weight (>10%), and non-neoplastic changes at the site of application and in the liver and testes. (Supported by Contract No. N01-ES-65406.)

897 SODIUM BROMATE TOXICITY STUDY USING Tg.AC AND p53 TRANSGENIC MICE.

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Sodium Bromate, a disinfection by-product (DBP) of drinking water chlorination/ozonization, was administered to Tg.AC and p53 transgenic mice to determine whether these transgenic strains could serve as models to identify the carcinogenic potential of DBP chemicals. In addition, the models may serve to rank families of DBP chemicals for toxicity. Tg.AC and/or p53 mice were exposed to sodium bromate in drinking water (0, 80, 400 or 800 mg/L) or by topical application (0, 64, 128, or 256 mg/Kg/day) for 26 (Core) or *gE* 39 (Special) weeks. No sodium bromate-related neoplastic lesions were observed in any of the groups. In the drinking-water study, Tg.AC and p53 mice exhibited decreased body weight (>10%) and changes in clinical pathology endpoints. Tg.AC mice also exhibited decreased survival and non-neoplastic changes in the thyroid gland, kidneys, pituitary gland, and testes/epididymides. In the dermal study, Tg.AC mice exhibited decreased body weight (>10%), clinical pathology endpoint changes, and non-neoplastic changes at the site of application, as well as in the thyroid gland, kidneys, spleen, liver, and testes. (Supported by Contract No. N01-ES-65406.)

898 BROMODICHLOROMETHANE (BDCM) TOXICITY STUDY USING Tg.AC AND p53 TRANSGENIC MICE.

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BDCM, a disinfection by-product (DBP) of drinking water chlorination/ozonization, was administered to Tg.AC and p53 transgenic mice to determine whether these transgenic strains could serve as models to identify the carcinogenic potential of DBP chemicals. In addition, the models may serve to rank families of DBP chemicals for toxicity. Tg.AC and/or p53 mice were exposed to BDCM in drinking water (0, 175, 350 or 700 mg/L), by gavage (0, 25, 50, or 100 mg/Kg/day) or by topical application (0, 64, 128, or 256 mg/Kg/day) for 26 (Core) or *gE* 39 (Special) weeks. No BDCM-related neoplastic lesions were observed in any of the groups. In the drinking water and gavage studies, mice exhibited increased liver weight and hepatocellular vacuolization (Tg.AC/p53 females); decreased liver weight and hepatocellular vacuolar changes (p53 males); and decreased kidney weight, nephropathy, and tubular dilatation (Tg.AC/p53 males). No site of application or systemic effects was observed for the Tg.AC or p53 mice in the dermal study. (Supported by Contract No. N01-ES-65406.)

899 USE OF C57BL/6 MICE IN CARCINOGENICITY BIOASSAYS.

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We present data from control groups of dietary carcinogenicity studies performed with C57BL/6 mice, for a total of 150 male and 150 female animals. The studies had a duration of 78 weeks. On termination of the studies the average survival was 92% for males and 90% for females. The most frequent causes of death were malignant lymphomas and generalised amyloidosis. During the study period average body weight gain for males was 12.2 g., and for females was 12.0 g. Average daily food consumption was 5.2 g (males) and 6.2 g (females); estimation was complicated by spillage of the diet, which was greater for females than males. Plasma levels of hepatic enzymes (ALAT, ASAT and ALP) were evaluated after 52 weeks and did not indicate any abnormalities. No palpable masses were observed at routine evaluation of the animals during the course of the studies. At histopathological examination of all organs, masses and macroscopic abnormalities, common non-neoplastic pathology findings included subcapsular spindle cell proliferation and ceroid degeneration in adrenals, vacuolation of brain white matter, necrotising inflammation of the Harderian gland, mononuclear cell aggregation in the renal pelvis, liver and pancreas, hepatic steatosis, histiocytosis of lymph nodes, atrophic changes in the ovaries, uterus and testes and generalised amyloidosis (adrenals, GI tract, heart, thyroids etc). Commonly observed tumours included malignant lymphomas (13% of males, 15% of females), hepatocellular adenomas (7% of males, 1% of females), and pituitary adenomas (8% of females). Remarkably few neoplastic lesions were found in the other organs and tissues, especially the lungs and the male and female reproductive organs. On the basis of the low and well-defined incidence of non-neoplastic and neoplastic lesions, it is concluded that the C57BL/6 mouse is a suitable model for carcinogenicity testing and that the present data will provide a useful basis for the future use of this strain in our laboratory.

900 COMPARISON OF THE INCIDENCE AND NATURE OF TUMORS IN AD LIBITUM AND RESTRICTED FEEDING REGIMENS FOR CHRONIC STUDIES USING THE SPRAGUE-DAWLEY RAT.

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Over the past years investigators working on life span calorie restriction aging and/or carcinogenicity studies have provided unequivocal evidence that the caloric restriction (about 25 to 40% of maximal caloric intake) supplemented with essential nutrients, retards the onset of aging and disease in rodents. Following these and similar findings there has been a move away from ad libitum feeding to restricted feeding in an attempt to increase animal survival by improving the overall health status of the animals. Previous analysis of the data collected at CTBR has shown that animals on these studies had improved survivability and had a lower body weight at the end of the dosing period. Lesion data from control animals from carcinogenicity studies using Sprague-Dawley rats from Charles River Canada were compared between restricted and ad libitum feeding regimens. The percentage incidences (total number of masses/total number of rats) of the major types of tumors/lesions were compared for evidence of any significant differences between the two feeding regimens and to determine any populations of tumors/lesions that were unique or predominately expressed in one of the two feeding regimens. Analysis of the data showed an increased incidence of spontaneous and endocrine tumors for ad libitum feeding when compared to restricted feeding (eg. pituitary adenoma); some significant differences in the incidences between the sexes of certain lesions within the same feeding regimen (eg. mammary gland ectasia); and that it was possible to identify tumors that were either unique or predominate to one of the two types of feeding regimens (eg. pituitary adenoma). In conclusion, restricted feeding results in a decreased incidence of spontaneous and endocrine tumors when compared to ad libitum feeding. Thus providing a more sensitive model for determining carcinogenic potential of a test compound.

901 THE EFFECT OF DIET AND ESTRADIOL ON ACI RAT MAMMARY CARCINOGENESIS.

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The ACI rat, an inbred cross of Copenhagen Irish and August strains, is a valuable model in the study of factors modulating susceptibility to mammary carcinogenesis. ACI rats are highly susceptible to mammary carcinogenesis when estrogen levels are chronically elevated. This study investigates the effect of a Western-style diet (high in fat and low in vitamin D and calcium) on estradiol-induced mammary oncogenesis. Adult female, ovary-intact ACI rats were implanted sc with a control 20 mg cholesterol pellet or a cholesterol pellet that also contained 1 or 3 mg of estradiol. Rats from each group were fed either the test Western-style diet or the control semipurified AIN-76A diet. After 10 weeks of study, one group of rats was sacrificed. There was a significant ($p < 0.05$) increase in liver wet weight for the estradiol-treated rats compared with the cholesterol-treated control rats, though not between either the two doses of estradiol or between the two diets alone. Estradiol caused a significant dose-dependent increase in pituitary wet weight that was not influenced by the diet. A second group was maintained until there was an appreciable mammary tumor burden or the rat became moribund. Rats implanted with the 3 mg estradiol pellet developed palpable mammary tumors at a median of 22 weeks. The diet did not influence estradiol-induced onset of palpable mammary tumors. Histological evaluation of tumors is being evaluated. Rats implanted with 1 mg estradiol and cholesterol are still under study. At this point in the study (32 weeks), it appears that the Western-style diet has little or no effect on the development of estradiol-induced mammary oncogenesis in this model. (Supported by ES05022, ES07148, GM44982)

902 EVALUATION OF THE CARCINOGENICITY OF BISPHENOL A TO RODENTS AND RELEVANCE OF FINDINGS TO HUMANS.

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Bisphenol A (BPA) is a monomer component of polycarbonate plastics and epoxy resins, which are used in numerous consumer products, including food-contact plastics. There has been considerable attention and scientific debate regarding the

relevance to humans of reported estrogenic actions of BPA. Conversely, there has been limited consideration of the carcinogenic potential of BPA. The issue of carcinogenicity is important in the regulation of chemicals for which there is potential for widespread exposure. An evaluation of the carcinogenic potential of BPA to humans was completed using metabolic data, genetic toxicity studies, long-term toxicity/carcinogenicity studies, and estimated consumer exposures. Two-year feeding studies in rats and mice administered diets containing up to 2, 000 and 10, 000 ppm BPA, respectively, provided no substantive evidence to indicate that BPA is carcinogenic to rodents. In addition, a battery of standard *in vitro* genetic toxicity assays and a GLP-compliant *in vivo* mouse micronucleus assay demonstrated a lack of genotoxic and mutagenic activity of BPA. Metabolism studies show that ingested BPA is rapidly glucuronidated and excreted. Furthermore, it is estimated that current uses of BPA, including those of food-contact materials, would result in very low human exposure. The toxicology data, taken together with the trivial exposure, support the conclusion that BPA is not a carcinogenic risk to humans.

903 ATRAZINE EXPOSURE *IN UTERO* INCREASES DIMETHYLBENZ[A]ANTHRACENE-INDUCED MAMMARY TUMOR INCIDENCE IN LONG EVANS OFFSPRING.

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Recently, we found that ATR exposure during mammary bud outgrowth (late gestation) delays normal postnatal epithelial progression in Long Evans (LE) to a greater extent than in Sprague-Dawley (SD) rats. To determine if ATR-induced developmental delays alter susceptibility of LE females to mammary carcinogen, we gavaged time-pregnant LE (10/treatment) and SD (11-15/treatment) dams with 0, 12.5, 25, or 50 mg ATR/kg body weight (2X/day) on gestation days 15-19. The female offspring were gavaged with dimethylbenz[a]anthracene (30 mg/kg) on postnatal day 45, a time when mammary glands have largely matured in controls. Animals were evaluated for palpable tumors weekly and killed 18 and 24 weeks later. SD offspring exposed to 0, 12.5, 25, and 50 mg ATR/kg (respectively) had overall mammary tumor incidences of 40, 25, 52, and 52% (X^2, p=0.15). Their tumor latency (20, 22, 21, and 22 weeks), multiplicity (2.25, 2.4, 2.9, and 1.5 tumors/tumor-bearing animal), and tumor volumes (3.05, 5.63, 1.81, and 1.08 cm3) were unchanged by gestational ATR. In contrast, LE offspring exposed to 0, 12.5, 25, and 50 mg ATR/kg had overall mammary tumor incidences of 41, 45, 62, and 60% (X^2, p=0.04). Although LE displayed no decrease in tumor latency (20.0, 21.6, 18.3, and 21.7 weeks), they had increased multiplicity (1.1, 1.5, 1.7, and 2.0) and larger tumor volumes (0.8, 0.2, 1.3, and 13.4 cm^3, P=0.04) following ATR *in utero*. Further, LE (but not SD) rats had significant increases in the percentage of animals with abnormalities in additional organs compared to controls (i.e, adrenal nodules, pituitary foci, ovarian cysts>2mm, and lymph node and spleen enlargement) at all ATR doses tested (p<0.05). Our data demonstrate that by delaying mammary gland development, gestational ATR exposure increases the susceptibility of the LE female to carcinogen, perhaps by extending the period of vulnerability. *(This abstract does not necessarily reflect EPA policy).*

904 CHLOROETHANE TOXICITY COMPARED TO STRUCTURALLY-RELATED CHLOROHYDROCARBONS.

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Chloroethane (CE) exposures at 15K ppm causes 86% uterine cancer incidence in B6C3F1 female mice(FM). CE's the most potent carcinogen in the NTP database by incidence. These cancers are (1) an uncommon tumor type, (2) highly metastatic, & (3) the cause of early FM deaths due to tumor load. Contrastingly, F344 rats similarly exposed were almost tumor free. This analysis examines uptake data into mice, rats, cats, guinea pigs, and human volunteers and is based on a peer reviewed a IRIS document and the open literature. Uptakes are similar among species. CE uptake relates to boiling point, Henry's constant, blood:air coefficient, and oil dissolution. SAR chlorohydrocarbons (CHCs)are compared with CE. Based on the physicochemical factors, CE shows the least tendency to get in and stay in the body of any CHC examined. Only high CE levels demonstrate any pharmacologic activity, analgesic (13K ppm) & anesthetic (35K ppm). The lowest recorded chronic pathologic level (5K ppm)is where oxidation is saturated and GSH is depleted especially in the adrenals, ovary, uterus, and liver. CE exposures > 5K ppm may be less detoxified and more available for toxicity. Hyperkinetic activity was observed only in the female mice (FM), but not in MM, MR or FR. Recursive running was observed only during CE exposure which likely produces a state of constant stress in FM. It's known human endometrial cancers can be caused by hyperadrenal activity. Thus, the unusual FM cancers may also be based on overt adrenal stimulation. While murine cancers are known only at the high CE above, low CE has never been tested. It's not likely that lower[CE]-which do not induce stress in FM-will cause uterine carcinogenesis. Re-doing the bioassay to include lower CE exposures, especially < 5K ppm, may indicate whether CE is a threshold carcinogen, or not. As it is, the present data set indicates CE has to considered possibly carcinogenic to humans. Nevertheless, long human use of CE as an surgery anesthetic, with few adverse reports, indicates the expectation of possible carcinogenicity may not hold upon further testing and analysis of CE.

905 ACRYLONITRILE IS A CARCINOGEN IN MALE AND FEMALE B6C3F1 MICE.

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Acrylonitrile is a heavily produced unsaturated nitrile, which is used in the production of synthetic fibers, plastics, resins, and rubber. Once considered a probable human carcinogen, IARC recently changed the classification for acrylonitrile to a possible human carcinogen. Acrylonitrile is a multi-site carcinogen in rats after exposure *via* gavage, drinking water, or inhalation. No carcinogenicity studies of acrylonitrile in a second animal species were available. The current studies were designed to assess the carcinogenicity of acrylonitrile in B6C3F1 mice of both sexes. Acrylonitrile was administered by gavage at 0, 2.5, 10 or 20 mg/kg/day, 5 days per week, for 2 years. A dose-related increase in the incidence of forestomach papillomas and carcinomas was detected in mice of both sexes with the incidences in the high dose reaching approximately 60% in both males and females. Higher incidences of focal or multifocal epithelial hyperplasia of the forestomach were observed in dosed mice. The incidence of harderian gland adenomas and carcinomas was also markedly increased in the acrylonitrile-dosed groups. In female mice, the incidence of benign or malignant granulosa cell tumors (combined) in the ovary in the 10 mg/kg dose group was greater than that in the vehicle control group, but because of a lack of dose-response, this was considered an equivocal finding. In addition, the incidences of atrophy and cysts in the ovary of the 10 and 20 mg/kg dose groups were significantly increased. The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) were significantly increased in female mice treated with acrylonitrile at 10 mg/kg/day for 2 years. This was also considered an equivocal result. In conclusion, these studies demonstrated that acrylonitrile causes multiple carcinogenic effects after gavage administration to male and female B6C3F1 mice for 2-years.

906 TERMINAL OBSERVATIONS IN SPRAGUE-DAWLEY RATS AFTER LIFETIME DIETARY EXPOSURE TO POTASSIUM PERFLUOROOCTANESULFONATE.

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Sprague-Dawley rats were given potassium perfluorooctanesulfonate (PFOS, 86.7 %) in the diet at 0, 0.5, 2, 5, & 20 ppm for up to 104 weeks. A recovery group received compound for 52 weeks. Results from interim sacrifices at weeks 4 and 14 were previously described. An interim sacrifice occurred at week 53 for histopathology, cell proliferation and PFOS determination. This report describes observations at 53 weeks through term. At 53 weeks, hepatic histomorphologic changes (without cell proliferation) and body weight effects were observed in 20-ppm animals. Through term, survival was significantly increased for males and body weights were significantly decreased for females at 5 and 20 ppm. Absolute and relative liver weights in males and relative liver weights in females were increased at 20 ppm. Liver toxicity was present at minimal doses of 2 ppm in males and 5 ppm in females. Hepatotoxicity was not present in 20-ppm recovery animals. For males, hepatocellular adenoma was increased for trend (p = 0.028) and pair-wise at 20 ppm (p = 0.046). Hepatocellular adenoma was not observed in the 20-ppm recovery group. Thyroid follicular cell adenoma was increased pair-wise for the 20-ppm recovery group (p = 0.028) which was outside the range of historical control values, yet there were no other indications of thyroid abnormality. For females, hepatocellular adenoma was increased for trend (p = 0.015) and pair-wise at 20 ppm (p = 0.039), hepatocellular adenoma/carcinoma was increased for trend (p = 0.006) and pair-wise at 20 ppm (p = 0.020), and mammary fibroadenoma was decreased for trend (p = 0.015) and pair-wise at 20 ppm (p = 0.024). The only hepatocellular carcinoma observed in the study was in a 20-ppm female.

907 TERMINAL OBSERVATIONS IN SPRAGUE-DAWLEY RATS AFTER LIFETIME DIETARY EXPOSURE TO N-ETHYL PERFLUOROOCTANESULFONAMIDO ETHANOL.

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Sprague-Dawley rats were given N-ethyl perfluorooctanesulfonamido ethanol (N-EtFOSE, 98.1 %) in the diet at 0, 1, 3, 30, 100 & 300 ppm for up to 104 weeks. A recovery group at 100 ppm received compound for 52 weeks. The 300-ppm groups

were terminated after seven weeks of treatment. Results from interim sacrifices at weeks 4 and 14 were previously described. An interim sacrifice occurred at week 53. This report describes observations at 53 weeks through term. At 53 weeks, decreased body weight, increased liver weight and hepatic histomorphologic changes (without cell proliferation), including a hepatocellular adenoma in a female, occurred at 100 ppm. Through term, survival was unaffected by treatment. Bodyweights were lower at 100 ppm. All liver effects occurred at 100 ppm, with the exception of cystic degeneration in 30-ppm males. Hepatotoxicity was not present in 100-ppm recovery animals. In males, thyroid follicular cell adenoma and combined thyroid follicular cell adenoma/carcinoma were increased for trend ($p = 0.017$ and 0.016, respectively) and pair-wise at 100 ppm ($p = 0.011$ for both); however, thyroid follicular cell carcinoma alone was not increased. For females, hepatocellular adenoma was increased for trend ($p=0.005$) and pair-wise for 100-ppm ($p = 0.012$) and 100-ppm-recovery ($p = 0.050$) groups, and combined hepatocellular adenoma/carcinoma was increased for trend ($p = 0.002$) and pair-wise for 100-ppm ($p = 0.007$) and 100-ppm-recovery ($p = 0.050$) groups. There was no increase for trend or pair-wise for hepatocellular carcinoma alone. The only hepatocellular carcinoma observed in the study was in a 100-ppm, terminal-sacrifice female. Significant negative trends and pair-wise decreases at 100 ppm occurred among females for mammary tumors (fibroadenoma, combined fibroadenoma/adenoma and combined fibroadenoma/adenoma/carcinoma). Trends for pituitary adenoma and combined adenoma/carcinoma were also negative in females.

908 CARCINOGENICITY OF DEPLETED URANIUM FRAGMENTS IN WISTAR RATS.

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The carcinogenicity of depleted uranium (DU) fragments embedded in muscle was determined in Wistar rats. Tissue reactions to DU fragments were compared to those of foreign-body fragments of tantalum (Ta) and to a radioactive compound, Thorotrast® (Th®). Flat squares or pellets of the metals were surgically implanted. Three sizes of DU fragments were used, 5x5x1.5 mm, 2.5x2.5x1.5 mm or 2.0x1.0 mm diam. Four fragments were embedded in each rat, 2 in each thigh muscle. Additional groups of rats received Th®injections *im.* as controls for radioactive materials, Ta implants (5x5x1.5 mm) as controls for foreign-body materials, or sham surgery as negative controls. Fifty rats per group were observed for life span for the onset, incidence, and types of reactions at the site. At death, all major organ systems were microscopically examined for lesions. Neither type nor amount of implant affected life span. Connective tissue capsules formed around the metal implants, but were much thicker around the DU fragments. Soft tissue tumors arose from some capsules 16 to 20 mo after implantation. Over 90% were malignant. Tumor incidence was significantly increased around the 5x5 mm squares of DU (18%) and the radioactive control, Th® (50%), when compared with the sham controls (0%). Incidence was slightly increased in rats with 2.5x2.5 mm DU squares (6%) and with 5x5 mm squares of Ta (4%). No tumors were seen in rats with the small DU pellets or in the sham controls. No increase in tumors was found in any other tissues. DU fragments of sufficient size cause local tissue reactions in the muscles of rats that can lead to localized soft tissue sarcomas. Implanting materials into the muscles of rats is sensitive for detecting potentially carcinogenic materials in humans. These findings do not indicate, however, that DU fragments are necessarily carcinogenic in humans. Funding:US Army Medical Research Development Command:MIPR# KVFM5529 with US DOE, Coop.Agreement #DE-FC04-96AL76406. Research conducted using SOT criteria and in AAALAC-accredited facilities.

909 INDUCTION OF LUNG TUMORS BY 7H-BENZO[c]FLUORENE.

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7H-BENZO(C)FLUORENE (B[c]F) is a major lung DNA adductor in mice treated with coal tar suggesting that B[c]F may be capable of inducing lung tumors. This study evaluates the tumorigenic potential of B[c]F using the A/J mouse model. Female A/J mice 7 weeks of age were administered BENZO(A)PYRENE (B[a]P) or B[c]F (100 mg/kg) by ip injection. These mice were fed 5001 rodent LabDiet for the remainder of the study. Groups of mice were also fed diets containing B[a]P (397 umol/kg diet), or B[c]F, (397 or 27 umol/kg diet). In addition, a mixture of twenty synthetic PAH known to be present in coal tar was also fed to mice in the presence or absence of B[c]F. A basal gel diet system was used to administer hydrocarbons within the diet. Mice were maintained on control or adulterated diets for 260 days. B[c]F administered ip (1.8 mg) induced multiple lung tumors in 92% of the treated mice, with an average of 4.0 tumors per mouse. 90% of the mice treated with 1.8 mg of B[a]P by ip administration also had lung tumors with an average of 6.7 tumors per mouse. However, the highest level of lung tumor induction was observed in mice fed 397 umol/kg diet B[c]F. A 100% tumor incidence and an average of 46 lung tumors per mouse was observed. In contrast, mice fed a diet containing 397 umol of B[a]P, had a 77% tumor incidence with an average of 1.4 tumors per mouse. Mice fed a 27 umol B[c]F per kg diet, or the mixture of synthetic hydrocarbons with or without B[c]F resulted in tumor incidences and multiplicity not different from controls. These results demonstrate that B[c]F is tumorigenic in lung of mice when administered by ip injection and in particular when fed to mice in the diet. These data, strongly suggest that B[c]F contributes to the potent mouse lung tumorigenicity previously demonstrated with coal tar when fed to mice . (This research was supported by funds from EPRI.)

910 COMBINATION EFFECTS OF HARMAN, NORHARMAN AND AMITROLE WITH NANO2 IN RAT HEPATOCARCINOGENESIS.

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N-Nitroso compounds, which induce cancers in various organs, may be formed endogenously after intake of amino compounds such as secondary amines and NaNO2 in combination. Harman and norharman, ÉÀ-carboline derivatives, which are found in some vegetables and in pyrolysis products. Amitrole had been used in pesticide. Therefore, the present study was performed to determine whether typical amino compounds (harman, norharman or amitorole) with NaNO2 might have modifying potential on liver carcinogenesis using a medium-term bioassay system. A total of 90 rats were divided into 6 groups, all given a single i.p. injection of DEN(200 mg/kg) at the commencement. After 2 weeks, the rats in group 1 and 6 received basal diet and diet containing amitorol(0.01%), respectively. Animals in groups 2, 3, 4 or 5 received diet containing basal diet, harman(0.05%), norharman(0.05%) or amitorole(0.01%) combination with 0.1% NaNO2 (given in the drinking water) for the remaining experimental duration. All rats were subjected to two-thirds partial hepatectomy at week 3 and survivors were sacrificed under ether anesthesia at week 8 for examination of livers immunohistochemically for glutathione S-transferase placental form (GST-P)-positive foci. Significant retardation of body weight gain and increment of thyroid weights were observed in rats treated with amitorole. Increase of kidney and/or liver weights were observed in the groups receiving harman or norharman. No modifying potential was evident in terms of numbers or areas of GST-P-positive foci in any groups. These results demonstrate that combinations of harman, norharman and amitrole with NaNO2 lack initiating or modifying potential for liver carcinogenesis in our medium-term bioassay system.

911 COMBINED INHALATION AND ORAL EXPOSURES OF MALE RATS TO CHLOROFORM ENHANCE CARCINOGENICITY IN THE KIDNEY AND THYROID.

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Community residents are usually exposed to hazardous chemicals such as chloroform through multiple routes of exposure in the living environment. For the purpose of assessing health effects of the exposed people, effects of combined inhalation and oral exposures to $CHCl_3$ on carcinogenicity/chronic toxicity were examined in male F344 rats. A group of 50 rats each was exposed through inhalation to 0 (clean air), 25, 50 or 100 ppm (v/v) of $CHCl_3$-vapor containing air for 6 hrs/day x 5 days/wk x 104 wks, and each inhalation group was given *ad libitum* either $CHCl_3$-containing drinking water of 1, 000 ppm (g/g) or the vehicle drinking water during the 104-wk period. Incidences of both the renal tumors (renal cell adenomas and carcinomas) and the pre-neoplastic lesion of atypical tubule hyperplasia in the kidney as well as incidence of the thyroid tumors (follicular adenoma and adenocarcinoma) increase in the combined exposure groups compared to the groups of the single exposure route. The incidences and/or severities of the non-neoplastic kidney lesions including karyomegaly of the proximal tubule, basophilic change and dilatation of tubular lumen and urinary glucose were also remarkably enhanced in the combined exposure groups, as compared to the groups of the single exposure route. The liver tumors were not observed, but the cases of both liver fatty change and inflammatory cell nest were increased primarily in the groups of oral administration. The non-neoplastic lesions of the nasal cavity were observed in the olfactory region, and affected primarily by the inhalation exposure. It can be concluded that the combined inhalation and oral exposures to $CHCl_3$ significantly

enhance the cacinogenicity of the kidney and thyroid in male rats. The non-neoplastic lesions of the liver and the nasal cavity seem to be affected, depending on the oral administration and the inhalation exposure, respectively. This study was contracted and supported by Japanese Ministry of the Environment.

912 PROMOTION OF THYROID FOLLICULAR CELL TUMORS IN RATS BY PREGNENOLONE-16α-CARBONITRILE (PCN).

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Pregnenolone-16α-carbonitrile (PCN) and Aroclor 1254 (PCB) both reduce serum thyroid hormone levels, but only PCN produces an increase in serum thyrotropin (TSH). PCN-mediated increases in TSH result in increased thyroid follicular cell proliferation and hyperplasia, which may represent early events on a morphological continuum leading to neoplasia. The purpose of this study was to assess whether PCN, which increases serum TSH, and PCB, which does not increase TSH, promote thyroid tumors in a 2-stage carcinogenesis model. Male SD rats were administered the thyroid tumor initiator diisopropanolnitrosamine (2.5 g/kg, sc), and after 7 days were fed control diet, diet containing 1000 ppm PCN, or diet containing 100 ppm PCB for 19 weeks. Body weight was unaffected by PCN treatment, however after 19 weeks of PCB treatment, body weights were reduced 21%. PCN treatment significantly reduced serum T4 through week 3 before recovery, whereas T4 levels following PCB treatment fell below detection limits by week 3 and remained drastically reduced through week 19. TSH concentrations in PCN-treated rats increased 3 fold at week 2, then declined to near control values at week 19. Unexpectedly, TSH concentrations reached nearly 2 times control after 1 week of PCB treatment, and were sustained until week 6. The incidence of thyroid follicular cell proliferative lesions, including cystic and follicular hyperplasia, cystic and follicular adenoma, and follicular carcinoma, was significantly increased following PCN treatment but not PCB treatment. PCB treatment caused an increase in thyroid carcinomas (4 of 22 rats) not associated with the proliferative-type lesions produced by PCN, despite an increase in TSH serum concentrations. In conclusion, PCN appears to promote thyroid tumors in a manner consistent with known effects of excessive TSH stimulation. However, thyroid carcinomas stemming from PCB treatment indicate that separate mechanisms exist for the production of thyroid cancer in rodents by chemicals classically considered microsomal enzyme inducers. (NIH grants ES-08156 and ES-07079)

913 PROMOTION OF TRIHALOMETHANE-INDUCED COLON ABERRANT CRYPT FOCI (ACF) BY A HIGH-FAT DIET.

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Bromodichloromethane (BDCM) and tribromomethane (TBM) enhanced neoplasia in the large intestine of rats when given by corn oil gavage; BDCM in the drinking water to male rats did not induce colon tumors, but did increase liver tumors. However, TBM and a mixture of THMs high in brominated trihalomethanes (THMs) induced colon neoplasia after 52 weeks. We found that brominated THMs induced ACF, putative preneoplastic lesions, in the colon when included in the drinking water of male rats. This study was undertaken to determine if the ACF can be promoted by a diet high in fat. Male F344/N rats were exposed to isomolar concentrations of the THMs: 0.5, 0.7, 0.9 and 1.1 g/l. of chloroform (TCM), BDCM, dibromochloromethane (DBCM) and TBM in the drinking water. Emulphor (0.25%) and 15 mg azoxymethane/kg ip (AOM) were the vehicle and positive control groups. Animals from each treatment group were fed the HF diet (Purina 5001; 19% fat). Groups from the emulphor, AOM, and BDCM treatments were given corn oil (0.5 ml/kg, po) 5 days/week. After 26 weeks the colons were examined for ACF. There was a statistically significant positive association between the ACF/colon (± SD) and the number of bromine atoms on the THM molecule: 0.25% emulphor (0), TCM (0.67 ± 0.75), BDCM (1.33 ± 1.11), DBCM (2.67 ± 2.36) and TBM (2.83 ± 2.34). AOM treated animals had 23.17 ± 7.75 ACF/colon. Corn oil did not increase the ACF/colon in the vehicle control or BDCM treatment groups, but did in the AOM group (32.5 ± 9.0). The HF fat diet was effective in promoting only the ACF induced by TBM (5.33 ± 2.26). The same result was found for the total number of crypts/colon. The data suggest a fundamental difference between TBM-induced ACF and those induced by the other THMs, a difference that might help to explain the ability of TBM, and not BDCM to induce colon neoplasia when administered chronically in the drinking water (This abstract of a proposed presentation does not necessarily reflect the views of the EPA)

914 THE INDUCTION OF COLORECTAL NEOPLASIA BY A MIXTURE HIGH IN BROMINATED TRIHALOMETHANES (THMS) ADMINISTERED IN THE DRINKING WATER TO MALE F344/N RATS.

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The THMs are the most widely distributed and concentrated of the chlorine disinfection by-products (DBPs). Bromodichloromethane (BDCM) and bromoform (TBM) administered in corn oil were found to increase the incidence of colon neoplasia in the rat. BDCM administered in the drinking water to male rats did not induce colon neoplasia. Since THMs occur together in finished drinking water, we initiated a study to evaluate the ability of a mixture high in brominated THMs (Krasner et al., J. Amer. Water Works Assoc., 81: 41-53, 1989) to induce colon neoplasia in male F344/N rats. The animals were exposed to mixtures of THMs at 10x, 100x, 1, 000x, and 10, 000x ambient concentrations. Water containing 0.25% emulphor (vehicle control), 15 mg azoxymethane/kg ip (positive control) and 477 mg TBM/L (= to the 10, 000X mixture) were included. Colon aberrant crypt foci (ACF; putative preneoplastic lesions) or neoplasia were scored at 13, 26 and 52 weeks. At 13 weeks the incidence (% of animals with ACF) was 100% (AOM), 63 % (TBM) and 88% (10, 000X THM). The 26 week incidence of ACF was 12% (0.25% emulphor), 100% (AOM and TBM), and 63%, 88%, and 88% in the 100X, 1000X, and 10, 000X THM groups respectively. At 52 weeks the incidence of ACF declined in all groups from the earlier time points. Pathologic analysis demonstrated an adenomatous polyp and hyperplasia (1/7 rats)in the 477 mg TBM/L and adenocarcinoma (1/7 rats)in the 10, 000X mixture treatment group. There are data to support the conclusion that ACF are preneoplastic and colorectal neoplasia is rare in F344/N rats; we consider this finding to be biologically significant. This is the first report of DBPs administered in drinking water to test animals inducing neoplasia in animals at a site concordant with the findings of epidemiological studies. (This is an abstract of a proposed presentation and does not necessarily reflect the view of the EPA.)

915 HISTOPATHOLOGICAL EFFECTS OF PROSTAGLANDINS AND ANALOGUES ON THE LIMITING RIDGE OF THE STOMACH IN RODENTS.

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The effect of oral exposure of rodents, notably rats, to certain prostaglandins of the E and F series was evaluated, particularly with respect to neoplastic potential. Exposures to misoprostol, 16, 16-dimethyl-PGE_2, and CL115 574, an older experimental PGE_1 analogue, in the range of 0.01 to 1 mg/kg bw/d are associated with proliferation of basal epithelial cells of the limiting ridge that separates the gastric stomach from the forestomach. Daily treatment for 10 days to several weeks, depending upon the prostanoid involved, results in gross observation of enlargement of the limiting ridge. Histological alterations include hyperplasia, acanthosis, and hyperkeratosis. Edema may also be present. In addition to specific effects on the limiting ridge, misoprostol, rioprostil, natural PGE_2, 16, 16-dimethyl-PGE_2, and methyl-PGE_2 also show trophic effects on the mucosa of the gastric stomach. Prostaglandin-associated pathology of the limiting ridge is non-neoplastic in nature as evidenced by the lack of progression in rat and mouse 2-year studies with misoprostol. Treatment of Sprague-Dawley rats with misoprostol at doses of 0.016 to 9.0 mg/kg bw/d for 52 weeks produced hyperkeratosis of the forestomach and limiting ridge that resolved following a 13-week recovery period. Reversibility was also shown for 16, 16-dimethyl-PGE_2 and CL115 574. In addition, there is no evidence that prostaglandins, natural or synthetic, possess genotoxic activity. The mechanism(s) by which prostaglandins exert a proliferative effect on the limiting ridge is not clear, but may involve an irritant and a direct trophic effect. Increased cell survival, retarded exfoliation, and the resultant accumulation of cells, may be postulated to potentially contribute to the effects observed. The role of the demonstrated cytoprotective effects of many PGE analogues is not known. Further elucidation of the mechanism(s)involved may shed light on the relevance to humans of the effects of prostaglandins on the forestomach in rodents.

916 EVIDENCE FOR THE CARCINOGENCITY OF DIAZOAMINOBENZENE BASED ON SHORT-TERM STUDIES.

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A series of short-term toxicity and metabolism studies on diazoaminobenzene (DAAB) provide clear evidence that DAAB can be designated as a carcinogen without performing a two-year bioassay. DAAB was nominated to the National

Toxicology Program (NTP) because of the potential for worker exposure from its use in laboratories, positive *Salmonella* mutagenicity data, its presence as an impurity in foods and cosmetics, and lack of adequate toxicological data. Structurally similar compounds to DAAB are carcinogenic, and it was speculated that DAAB would be metabolized to benzene (BZ) and aniline (AN). Recently reported metabolism studies confirmed that DAAB is rapidly metabolized to BZ and AN in mice and rats, with the predominant circulating metabolites being common to those of BZ and AN (Mathews and De Costa, 1999). In a later study, phenyl radicals were shown to be produced both *in vitro* and *in vivo* during the reductive metabolism of DAAB to BZ and AN (Kadiiska *et al.*, 2000). Based on these studies, it was expected that a combination of adverse effects consistent with BZ and AN would be observed *in vivo*. 16-Day dermal exposure studies in mice and rats indicated that DAAB induced a methemoglobinemia and Heinz body anemia similar to that induced by AN. Some observed toxicological effects possibly related to BZ included splenic lymphoid follicle atrophy and hematopoietic cell proliferation. To further explore similar toxicological effects of DAAB and BZ, mice were gavaged with BZ or DAAB once a day for two days and assessed for the frequency of micronuclei in bone marrow erythrocytes. DAAB induced a similar number of micronuclei as BZ, but at a 10-fold lower dose suggesting that AN, and possibly the phenyl radicals formed during the reductive metabolism of DAAB, induce micronuclei and may act in a synergistic manner with BZ. In conclusion, because DAAB is metabolized to the carcinogens, BZ and AN, and has similar toxic effects to these compounds *in vivo*, it should be regarded as a carcinogen.

917 THE NORWEGIAN NATIONAL REGISTRY OF SEVERE ALLERGIC REACTIONS TO FOOD.

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There is a public perception that food allergy is very common. However, in some studies, it has been possible to diagnostically verify only 1 out of 5 to 10 self-claimed cases. The prevalence of food allergy in Norway is unknown, and little is known about risk groups, foods, and the circumstances under which the reactions occur. Diagnostic follow-up and patient care appear to vary greatly. Patients often feel that their problems are neglected. As part of the Government Action Plan against asthma, allergy, and indoor air-related diseases, funding has from year 2000 on been provided for a national reporting system for severe allergic reactions to food. The purpose is to obtain data for the incidence and other information about severe allergic reactions to food in Norway, in the first instance as experienced in the health care system-patient interface before more extensive diagnostic work has been performed. Further, free food allergen analysis in relation to reported cases is offered, and also standard diagnostic and advanced serum analysis including a food allergen macroarray with more than 100 food allergens. The reporting system also has a surveillance function in relation to food labelling and allergen occurrence in presumably "safe" foods. The reporting system was launched July 1, 2000. Information material, single-page reporting forms and informed consent forms were mailed to all doctors (6500) with relevant practices, clinics and emergency wards. By December 31, 2000, after 6 months, 42 cases had been reported. Peanuts were found to be the most common food allergen causing specific IgE sensitization. There were about equal numbers of males and females among the cases. The age distribution of the reported severe food allergy reaction cases showed a very marked peak for cases with age 20-30 years, and to a lesser extent small children and teenagers. Data including year 2001 results will be presented.

918 EVALUATION OF THE HEALTH ASPECTS OF METHYL PARABEN IN SUPPORT OF AN ACCEPTABLE DAILY INTAKE IN HUMNAS.

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Methyl paraben (CAS No. 99-76-3) is a methyl ester of p-hydroxybenzoic acid. It is a stable, nonvolatile compound used as an antimicrobial preservative in foods, drugs, and cosmetics for over 50 years. Methyl paraben is readily and completely absorbed through the skin and from the gastrointestinal tract. It is hydrolyzed to p-hydroxybenzoic acid, conjugated and the conjugates are rapidly excreted in the urine. There is no evidence of accumulation. Acute toxicity studies in animals indicate that methyl paraben is practically non-toxic by both oral and parenteral routes. In a population with normal skin, methyl paraben is practically nonirritating and nonsensitizing. In chronic administration studies, no observed effect levels (NOEL) as high as 1050 mg/kg have been reported and a no observed adverse effect level (NOAEL) in the rat of 5700 mg/kg is posited. Methyl paraben is not carcinogenic or mutagenic. It is not teratogenic or embryotoxic and is negative in the uterotrophic assay. The mechanism of cytotoxic action of parabens may be linked to mitochondrial failure dependent upon induction of membrane permeability transition accompanied by the mitochondrial depolarization and depletion of cellular ATP through uncoupling of oxidative phosphorylation. Parabens are reported to cause contact dermatitis reactions in some individuals exposed cutaneously. Parabens have been implicated in numerous cases of contact sensitivity associated with cutaneous exposure, however the mechanism of this sensitivity is unknown. Sensitization has occurred when medications containing parabens have been applied to damaged or broken skin. Allergic reactions to ingested parabens have been reported, although rigorous evidence of the allergenicity of ingested paraben is lacking. Based on the history of the safe use of methyl paraben and literature reviewed, an increase in average daily intake (ADI) to 55 mg/kg/day for methyl paraben is justified.

919 UP-REGULATION OF GASTRIC ALDEHYDE DEHYDROGENASE (ADH) IN RATS BY SHORT TERM FEEDING OF 2, 4-HEXADIENAL (HX).

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Many foods and food products contain HX (an aldehyde) either naturally or as additives. Low level of exposure to HX is widespread. Long term feeding of HX led to high incidence of forestomach cancers in young rodents. ADH, being a key enzyme for metabolizing aldehydes, would play a role in modulating the biological activity of ingested HX. We studied gastric ADH in young rats to see whether this enzyme acts on HX and whether short term feeding of HX affects the gastric ADH activity. Both the rat fore- and glandular stomach contained ADH activity and preferred using HX > acetylaldehyde > formaldehyde as the substrate. In postweaned rats, the forestomach has a higher level of ADH compared to the glandular stomach. Feeding of HX to ~26 day old rats for 5 days led to minimal changes in stomach weight, but increased ADH activity in the forestomach (3X of control). ADH activity did not change significantly in the glandular stomach, esophagus, liver or small intestine of the treated rats. The increase in ADH activity was dependent on the duration of feeding. Moderate increase was found after 2 days feeding of HX (100 mg/Kg body wt. daily). The activity increased to 3-3.5X of controls after 4 to 5 days of feeding. Thus, HX selectively up-regulated ADH activity in the forestomach but not in any other tissues of the GI tract. This suggests HX specifically targets the forestomach. The present observation also indicates the occurrence of underlying cellular changes that eventually might manifest as carcinogenesis in the forestomach. The exact changes are unknown and currently under study.

920 EXTRACTS OF GRAPES AND BERRIES INHIBIT OXIDATION AND MUTATION BY PEROXYNITRITE IN THREE MODEL SYSTEMS.

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Oxidative and nitrosative damage plays a major role in the etiology and progression of a number of diseases involving inflammation. Experimental evidence exists that dietary sources of antioxidants can retard or prevent this type of damage. The objective of this study was to develop simple model systems to study the effects of dietary sources of antioxidants toward the actions of the powerful oxidative and nitrosative substance, peroxynitrite. With these systems, we tested the ability of grape-derived beverages, extracts of white, red and black grapes as well as raspberries, blueberries, and cranberries, to inhibit oxidative/nitrosative processes including chemiluminescence, DNA nicking and plasmid infectivity of E. coli. The results demonstrate that grape-derived beverages (wines and grape juice), and grape and berry extracts inhibited luminol-dependent chemiluminescence induced by SIN-1 (which breaks down into peroxynitrite) over a wide range of dilutions. Generally, the darker the test material, the more potent the action. Peroxynitrite was incubated with the G-Wiz luciferase high expression plasmid which contains a kanamycin resistance marker and the luciferase gene. Extracts of grapes and berries were effective inhibitors of plasmid DNA nicking. In another set of experiments, incubation of the plasmid with peroxynitrite caused mutation so that it could not infect E. coli strain DH1alpha. Extracts of blueberries, black grapes, dried cranberries and golden raisins were the only preparations effective in reversing the inhibition. In conclusion, antioxidant potencies of the preparations were not equivalent across the three systems; in general the darker the extract the greater the potency.

921 MECHANISM OF APPETITE SUPRESSION BY A NOVEL, NATURAL HYDROXYCITRIC ACID.

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A growing body of evidence demonstrates the efficacy of *Garcinia cambogia*-derived natural (-)-hydroxycitric acid (HCA, CitriMax®) in weight management by curbing appetite and inhibiting fat synthesis. In a previous study, we showed that HCA

can increase the release/availability of radiolabeled 5-HT ([^{3}H]-5-HT from rat brain cortex (FASEB J. 15: Abs. 207.13, 2001), and this could explain HCA's reported role in appetite suppression. Although HCA has been reported to suppress appetite and reduce food intake in experimental animals and humans, its mechanism of action is not fully understood. In the present study, we investigated the possibility that the observed effect of HCA on [^{3}H]-5-HT release from isolated rat brain cortex could be due to an action on 5-HT uptake. Isolated rat brain cortex slices were initially incubated in oxygenated Krebs solution for 20 minutes and then transferred to buffer solutions containing [^{3}H]-5-HT for different time intervals. In test experiments, tissues were exposed to HCA (10 μM – 1 mM), fluoxetine (100 μM) and cloimipramine (10 μM). Uptake of [^{3}H]-5-HT was expressed as d.p.m./mg wet weight. A time-dependent uptake of [^{3}H]-5-HT occurred in cortical slices reaching a maximum at 60 mins. HCA, fluoxetine and/or clomipramine inhibited the time-dependent uptake of [^{3}H]-5-HT. At 90 mins, HCA (300 μM) caused a 20% decrease whereas fluoxetine plus cloimipramine inhibited [^{3}H]-5-HT uptake by 30%. Thus, HCA can inhibit [^{3}H]-5-HT uptake (and increase 5-HT availability) in isolated rat brain cortical slices in a manner similar to those of selective serotonin reuptake inhibitors (SSRI) and may have the antidepressant properties of fluoxetine. In addition, safety studies including acute oral toxicity, dermal toxicity, dermal irritation and eye irritation, were conducted to determine HCA's safety profile. The LD_{50} of HCA was found to be greater than 5000 mg/kg when administered orally, and other results were encouraging. Taken together, these results demonstrate that HCA is safe and helps suppress appetite.

922 BUTYLATED HYDROXYTOLUENE INHIBITS AFLATOXIN B_1 ACTIVATION AND RELATED METABOLIZING ENZYMES IN TURKEY LIVER MICROSOMES.

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The mycotoxin aflatoxin B_1 (AFB_1) is a potent hepatotoxin, and a common contaminant of foods and feeds. Turkeys are extremely susceptible to the toxic effects of even small amounts of this mycotoxin, a condition we have shown to be due to a combination of efficient hepatic cytochrome P450 (CYP)-mediated activation and deficient glutathione S-transferase (GST)-mediated detoxification of AFB_1. We recently demonstrated that butylated hydroxytoluene (BHT) protects against nearly all of clinical signs of aflatoxicosis in turkeys, an effect that is not apparently mediated by induction of GSTs. The purpose of this study was to determine the possible mechanism of BHT-mediated protection of aflatoxicosis in turkeys. Here we show that BHT is a competitive inhibitor (Ki = 5.38 mM) of AFB_1 activation to the AFB_1-8, -9-epoxide (AFBO) in liver microsomes prepared from male white turkeys. Furthermore, BHT inhibited microsomal ethoxyresorufin O-deethylase (EROD), methoxyresorufin O-demethylase (MROD), and pentoxyresorufin O-depentylase (PROD), indicator activities of CYPs 1A1, 1A2 and 2B1, respectively, in a dose-related manner. That 1A activities were the most sensitive to BHT inhibition supports an earlier observation that AFB_1 activation in turkey liver is largely mediated by a CYP 1A homolog. It is therefore possible that the observed chemoprotective properties of BHT in turkeys may be due, at least in part, to its ability to inhibit hepatic AFB_1 activation to reactive toxic intermediate(s). (Supported by USDA-NRI grants 97-3081 and 98-3754).

923 CHEMICAL CHARACTERIZATION OF CHROMIUM PICOLINATE MONOHYDRATE.

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NTP is conducting rodent 90-day toxicity and 2-year carcinogenicity studies of chromium picolinate which is a nutritional supplement that is being marketed for weight loss. The present work describes the methods and results of studies to confirm the identity of the bulk chemical and to estimate its purity and stability. IR, ^{1}H-NMR, and XRD spectroscopy were consistent with the structure and identity of the bulk chemical as chromium picolinate monohydrate $Cr(Pic)_3 \bullet H_2O$. LC-ESI/MS revealed the presence of an intense ion at *m/z* 436 consistent with the $[M + H]^+$ ion of $Cr(Pic)_3 \bullet H_2O$. The presence of one mole of water in the complex was also revealed by Karl Fischer titrimetry. Elemental analysis for carbon, hydrogen, and nitrogen (98.5%, 94.9%, and 97.3%, respectively, of theoretical values), and ICP-AES analysis for total chromium(103 ± 2% of the theoretical value) supported the identity of the bulk chemical as $Cr(Pic)_3 \bullet H_2O$. Total impurities detected by PIXE were <0.5%. Using LC-ICP/MS, the maximum concentrations of either free Cr(III) or Cr(VI) in the bulk chemical were <0.025%. Purity evaluation using HPLC with UV detection at 265 nm revealed a major component at ~96%, one impurity present at 2.1%, and an additional five impurities each present at levels below ~0.7%. The presence of impurities was not unexpected since several chromium:picolinate complexes can arise from the aqueous phase equilibria of this compound. LC-ESI/MS revealed that many of the components present in synthetic mixtures of chromium:picolinate complexes were also present as impurities in the bulk chemical, but unequivocal identification of the components was not possible due to the lack of pure authentic reference standards. Stability studies revealed that the bulk chemical was stable for at least two weeks at temperatures up to 60°C, demonstrating that storage at room temperature is acceptable for long-term studies.

924 THE EFFECT OF TRIBUTYLTIN ON MICROBIAL INFECTIONS OF MICE.

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Tributyltin compounds (TBTs) are immuno-toxic chemicals, having been artificially introduced to marine environments. Recently, the contamination of more than 1ppb TBTs has been found in most of fishes yielded in Japanese Fish Market. This suggests continuous exposure of TBTs to the Japanese people through direct ingestion of fish or other sea foods. In the present study, the effect of tributyltin chloride (TBTCl) by oral exposure was examined on host resistance of ICR off-spring mice against challenge of microbial infection. Off-spring mice received TBTCl from lactation of mother mice that had taken drinking water with 0, 5, 15 or 50 ppm TBTCl. On werning time (day 21), they were infected with *Listeria monocytogenes* or *Candida albicans* . The host resistance to these microbial infections were estimated by the numbers of recovered microbes in spleen at the day 2, 4 and 6 for *L. monocytogenes* infection, and by those in kidney and brain at the day 43 for *C. albicans* infection, respectively. The Listeria was detected in spleen throughout the experiment obtained from off-spring groups that were lactated by mother given TBTCl at the concentration of 50 ppm. Besides, Candida was also found in the brain of off-spring groups given 50 ppm TBTCl, while no Candida was detected in the control group without TBTCl. In addition, FACS analysis at the weaning point showed that TBTCl induced the slight decrease of CD8+ T cell population in spleen. These results suggest that the exposure of the extremely low levels as 50 ppm TBT through lactation by mother leads to decline of host resistance against microbial infection in off-spring.

925 YEAST ESTROGEN SCREEN TO TEST FOR ESTROGENIC ACTIVITY OF VARIOUS INFANT FOODS.

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High intake of estrogenic compounds has raised concern about potential adverse effects on reproduction and development, especially in infants as susceptible subgroup of the population. Intake estimates are usually based on analytical chemistry and take not into account actual biological activity. Yeast stabely transfected with the estrogen receptor and a reporter construct is a commonly used assay to screen for estrogenic activity of chemical compounds. The purpose of our study was to use this screening tool, expressing the human ERα, to test for estrogenic activity in various infant foods. Soy infant formula, containing isoflavones, are compared to milk-based formula, human and cow's milk, which may contain phytoestrogens as well as steroidal estrogens. We screened the extracts of the various foods as well as directly the diluted or reconstituted foods in comparison to the main conjugated and free isoflavones as well as steroidal estrogens. Overall activity of soy formula was low compared to steroidal estrogens and isoflavones. No activity was detectable in any of the other foods under the conditions of our test system. Extracts of the foods are easier to analyze, reconstituted milks tested directly influence the cell growth and interfere with OD measurements. Since the compounds tested in this estrogen screen have different chemical structures with different physico-chemical properties, we tested the penetration of test compounds into the yeast cell, using C14-labelled estradiol and C14-labelled genistein. In both cases the rate of penetration was below 1% of the total amount added after over night incubation. Although a fairly easy and simple to use system, the yeast estrogen screen allows only for relative comparison of overall estrogenic activity of food extracts and for screening purposes of pure compounds. Conclusions on potential health effects of high intake of dietary estrogens cannot be drawn based on results from such an assay. More information on *in vivo* uptake, metabolism, distribution and internal exposure in target organs is necessary.

926 ASSESSMENT OF THE ALLERGENICITY OF KNOWN HUMAN ALLERGENIC AND NON-ALLERGENIC FOOD PROTEINS IN MICE.

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No validated animal model exists which can predict protein allergenicity. A model has been proposed by Dearman et al. that has exhibited divergent antibody isotype responses (IgE vs IgG2a) in female BALB/c mice exposed IP to a known human allergen, ovalbumin (OVA) vs a non-allergen, BSA. To examine the reproducibility

and predictability of the proposed model, two additional proteins, potato acid phosphatase (PAP) and β-lactoglobulin (β-lac), and two additional strains of mice, C3H/HeJ and A/J, were evaluated for allergic responses. Ten female mice of each strain were IP injected once a week for two weeks with 1, 2 or 5% BSA, OVA, β-lac, or PAP in PBS buffer. One week after the last injection, animals were euthanized and blood was collected from the vena cava. Serum antigen-specific IgG, IgG1, and IgG2a were determined by ELISA and bioactive IgE to each protein was determined by PCA in naïve rats. Serum antigen-specific IgG was observed in a dose dependent manner for all four proteins tested. Overall, BSA demonstrated the highest titer of IgG followed by OVA, β-lac, and PAP. Unlike the results of the Dearman study, bioactive IgE was not observed in BALB/c mice treated with OVA, or with β-lac or PAP, a human food allergen and non-allergen, respectively. A/J mice treated with 2 and 5% OVA demonstrated clinical signs of anaphylaxis. Bioactive IgE was observed in A/J mice treated with OVA at all dose levels. Bioactive IgE was also observed in A/J and C3H/HeJ mice treated with PAP and β-lac. The strongest IgE responses in terms of the number of responder mice and antibody titer were observed in A/J mice treated with 2% OVA or 1% PAP. Bioactive IgE was not observed in any strain treated with BSA. These results suggest that the production of bioactive IgE is dependent on the genetic background of the mice exposed and the dose of protein that is administered. The IgG response to each protein is more uniform and may be a useful means of determining exposure to a test protein.

927 CHLORINATED HYDROCARBON AND CHLORINATED ORGANOPHOSPHATE RESIDUES IN HORSEMEAT FROM THE FSIS NATIONAL RESIDUE MONITORING PROGRAM, 1990–1999.

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The National Residue Program (NRP) conducted by the USDA Food Safety and Inspection Service (FSIS) includes a comprehensive testing program for residues of pesticides, drugs, and other chemical contaminants in meat, poultry, and egg products. Samples are taken statistically so that inferences can be made on the overall national incidence of residues. Since 1990, there has been a large decline in the number of horses slaughtered for food in the US The number of horses slaughtered in federally inspected establishments in 1999 is only 20% of the number slaughtered in 1990 (60, 138 in 1999 *vs.* 306, 771 in 1990). Using a methodology that simultaneously detects and quantitates 36 different CHC's/COP's, FSIS conducted 3, 490 analyses in horses between 1990 and 1999. During this period only 13 violative residues were found, resulting in a violation rate of 0.4%. The violative findings consisted of the following; five coumaphos violations; three dieldrin violations; two heptachlor violations; and three PCB violations. In addition, FSIS detected 339 non-violative positive residues (i.e., residues present at levels less than or equal to the tolerance or action level). The compounds found at non-violative levels were: dieldrin, DDT, heptachlor, lindane, methoxychlor, hexachlorobenzene, mirex, endosulfan, PCB's, chlorpyrifos, coumaphos and endrin. Since 1972, the United States Environmental Protection Agency has banned all uses of DDT, except for public health emergencies. Nevertheless, non-violative residues of DDT were found every year except 1990, with the highest numbers in 1993 and 1998 (40 and 25, respectively). This can be explained by the environmental persistence of DDT residues that were introduced by usage prior to the 1972 ban. These findings indicate the value of the FSIS NRP in assessing and communicating human exposure to chemical residues, and in maintaining consumer confidence in the safety of the US food supply by demonstrating that the incidence of violative levels of these contaminants is very low.

928 RELATIONSHIP OF TRICHOTHECENE STRUCTURE TO COX-2 INDUCTION IN THE MACROPHAGE MODEL.

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Cyclooxygenase-2 (COX-2) is a crucial rate-limiting enzyme in the production of proinflammatory eicosanoids. Previous studies have shown that deoxynivalenol (DON) induced COX-2 expression by promoting transcriptional activity and mRNA stability *via* mitogen activated protein kinase (MAPK) signaling pathway. Based on this model, representative members of the three major trichothecene families (A, B, and D) were screened for the inducibility of COX-2 expression using murine macrophage, RAW264.7 cells. When treated with the 20% inhibitory doses (ID_{20}) based on MTT assay, Type B trichothecenes including DON, 15-acetylDON, 3-acetylDON and fusarenon-X (FX) were potent inducers of COX-2 expression whereas Type A and D had little effect on COX-2 expression. To assess the effect of the trichothecenes on transcriptional and post-transcriptional activity, luciferase reporter vectors containing 5' promoter or 3'-untranslated region (UTR) of COX-2 gene were transfected into RAW 264.7 cells and the reporter activities were compared following the trichothecene treatment. Typically, type B trichothecenes enhanced the COX-2 promoter activity and UTR-linked mRNA stability whereas Type A and D did not affect the reporter activities. The COX-2-inducing trichothecenes also activated MAPK signaling pathways, and ERK or p38 inhibitor significantly reduced the COX-2 induction. In contrast, the dominant negative JNK expression did not diminish the trichothecene-induced COX-2 expression although JNK was activated by the four toxins. Additionally, pyrrolidine dithiocarbamate, an inhibitor of nuclear factor-kB (NF-kB) activation, suppressed the trichothecene-induced COX-2 expression. Taken together, type B trichothecenes including DON, its derivatives and FX are direct strong inducers of COX-2 gene and the induction is due to both enhanced transcription and mRNA stabilization. Furthermore, MAPK and NF-kB signaling pathways mediate the trichothecene-induced COX-2 expression, which may contribute to the type B trichothecene immunotoxicity.

929 SAFETY EVALUATION OF EPHEDRA DIETARY SUPPLEMENTS.

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The safety of ephedra/ephedrine alkaloids has been studied in animals and humans. A series of clinical investigations have been done with obese but otherwise healthy volunteers receiving oral ephedra or ephedrine over periods of 10 d to 26 m. The range of total doses within these studies was from 50 to 150 mg/d (the LOAEL) in 1 to 3 doses. Monitoring data for adverse effects identified the NOAEL of 90 mg/d; reported symptoms, such as chest pain, palpitation, irritability, nausea and constipation were similar among supplement and placebo groups. Carcinogenicity studies in mice and rats demonstrated a lifetime NOAEL of 9 mg ephedrine/kg bw/d. The only finding at this dose was decreased body weight gain, which increased, or had no effect on, survival. These lifetime rodent studies showed that there were no long-term, cumulative effects that may not be detected in clinical studies. Using the results obtained from these investigations, application of the National Academy of Sciences Upper Level (UL) Model derived a UL of 90 mg/d of ephedrine alkaloids in ephedra for a generally healthy population. This assessment included the use of an Uncertainty Factor of 1, applied to the human NOAEL. This UF is based on consideration of the pharmacokinetics of ephedrine, use patterns, duration of expected use, animal and clinical studies, without consideration of the voluntary database of adverse event reports that we found to be generally unreliable and insufficient to draw any conclusion about the safety of ephedra dietary supplements.

930 13-WEEK FEEDING STUDY IN RATS FED GRAIN FROM YIELDGARD ® EVENT MON 810 CORN.

B. R. Dudek, B. G. Hammond, J.K. Lemen, M. A. Nemeth and J. D. Astwood. *Monsanto Company, St. Louis, MO.*

As part of the safety assessment of food/feed derived from genetically modified crops, it is important to evaluate unintended consequences owing to the insertion of the transgene into the genome or expression of the resulting trait. In combination with other approaches such as compositional evaluations and nutritional assessments in farm animal feeding studies, a subchronic feeding study in rats may address such unintended effects. This study compared the responses of rats fed diets containing grain from YieldGard (YG) corn to rats fed (1)diets containing grain from near-isogenic control (C) (non-transgenic) corn and (2)a population of diets containing grain from commercial reference corn (RC) hybrids. Male and female CD IGS rats (20/sex/group) were fed one of the following diets for 13 weeks: 11 or 33% (wt/wt) (YG) or (C) grain; 33% (RC) grain (6 commercial lines were tested). There were a total of 10 groups/400 rats in the study. Diets containing 11% YG or C grain were supplemented with 22% grain (non-transgenic commercial corn) to bring the total corn grain to 33%, consistent with other diets. Laboratory rat feed contains corn grain at 33%. Diets were balanced to be nutritionally equivalent. Animals were observed daily; body weights and food consumption were recorded at least weekly. At 5 and 13 weeks, blood and urine were collected from 10 rats/sex/group for clinical evaluations. After 13 weeks, all animals were sacrificed, necropsied and selected tissues weighed and examined microscopically. Body weights and food consumption were comparable for all groups. Clinical parameters were comparable across groups with few exceptions. The few differences were not considered biologically meaningful as they were either not dose related, or were not consistently different from both control groups. In conclusion, rats fed grain from YieldGard corn responded similarly to rats fed control grain. This conclusion was consistent with previous studies showing that YG corn grain is compositionally and nutritionally equivalent to traditional corn.

931 ONE YEAR TOLERANCE STUDY WITH LUTEIN AND ZEAXANTHIN IN CYNOMOLGUS MONKEYS.

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A one year tolerance study was performed in Cynomolgus monkeys to evaluate the toxicity of lutein and zeaxanthin administered as 10% beadlet formulations by gavage at 0, 0.2 and 20 mg/ kg/ day (pure Carotenoid) to groups of 2 male and 2 female Cynomolgus monkeys. Additionally, 1 male and 1 female animal at 20 mg/kg/day were sacrificed after 6 months of treatment. All animals survived the treatment period. All animals in the 20 mg/kg/day group of lutein and zeaxanthin showed orange/yellow discolored feces from day 2 of the study onwards. There was no effect on overall mean body weight gain and on overall group mean food intake in animals of all treated groups. There were no changes in electrocardiography and blood pressure data, which could be regarded as being related to treatment. There were no treatment related changes in hematological and serum clinical chemistry parameters, nor changes in urine parameters. There were no organ weight changes, which could be associated with treatment. Most of the animals showed dark-yellow colored mesenteric fat at interim sacrifice and gold-yellow mesenteric fat at the terminal sacrifice. This finding is considered to be related to the color of the compounds. At histopathological examinations no treatment-related findings were reported. Ophthalmoscopic examinations, electroretinography and eye pathology examinations were performed. There was no clinical and no morphological evidence for treatment-related adverse changes in the eyes of Cynomolgus monkeys during or after 52 weeks treatment with lutein or zeaxanthin. Specifically, there was no evidence for crystal formation in the eyes of treated monkeys. Administration of the test article resulted in dose-dependent increase of lutein or zeaxanthin (HPLC determinations) in plasma, liver, macula lutea and peripheral retina. Extrapolation of results in Cynomolgus monkeys to man is reliable, especially regarding eye findings, and it was concluded that lutein and zeaxanthin are safe products.

932 AN ASSESSMENT OF THE GENOTOXICITY AND SUB-CHRONIC TOXICITY OF PHYTOSTEROL OXIDES.

L. J. Lea, A. M. Wolfreys and P. A. Hepburn. *Approval and Support, Unilever SEAC, Bedford, United Kingdom.*

Phytosterols are structurally very similar to cholesterol and their cholesterol-lowering ability has been well documented since the 1950's. Added to vegetable oils spreads as the fatty acid esters they have been marketed as a cholesterol lowering functional food. The safety of phytosterol-esters has been well established and has led to regulatory approval of vegetable oil spreads enriched with phytosterol-esters in over 20 countries world wide. Phytosterol-esters also have GRAS status in the US. Phytosterols are very stable molecules and oxidation only occurs at low (ppm) levels even under extreme heating conditions. As such conditions could occur during shallow frying with phytosterol-ester enriched spreads safety data has been generated on the breakdown products, phytosterol oxides. The genotoxicity and subchronic toxicity of a mixture of phytosterol oxides was assessed in a series of *in vitro* mutagenicity assays and a 90-day feeding study in rats. There was no evidence of mutagenicity in either a bacterial or mammalian cell assay and an additional assay designed to detect aneuploidy was also negative. In the 90 day feeding study in rats the same phytosterol oxide mix was added to the diet of Wistar rats at levels of 0.2, 0.6 and 1.6% w/w. A number of minor toxicological findings were observed in the high dose group particularly in female animals. These consisted of reduced body weight gains, small changes in a number of clinical chemistry and haematology parameters and increased liver weights. However, no histopathological changes were observed. Based on these findings the NOAEL (no observed adverse effect level) was set at the mid dose level of 0.6%w/w phytosterol oxides. This was equivalent to an intake of the phytosterol oxide mix of 425mg/kg/bw/day for males and 481mg/kg/bw/day for females.

933 RESPONSIVENESS OF HEAT SHOCK PROTEIN GENES TO A RANGE OF HERBAL SUPPLEMENTS.

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The genes that encode heat shock proteins (HSP) are coordinately regulated in response to acute exposure of cells to a range of physiological and environmental factors such as heat shock, oxidative stress, and anti-inflammatory drugs. Many herbal supplements are marketed as anti-oxidants and anti-inflammatory agents. The present study was undertaken to test activation activity of 14 commonly used herbal supplements on reporters HSP27, 70 and 90b. Co-transfection experiments in 293T cells were conducted with an HSP reporter and the pRL-TK plasmid (transfection efficiency control). After a 12-hr incubation, the transfected cells were treated with an herbal supplement or vehicle for 24 hr. Luciferase activity was determined and used as an indicator for supplement-mediated regulation on the HSP reporters. Majority of the supplements exhibited activity toward the HSP90b reporter. In contrast, only one third of them exerted activity toward the HSP27 reporter. The magnitude of activation of the HSP27 reporter was less that that of the HSP90b. In addition, both green tea and Kava-kava caused activation of all three reporters to a similar extent. It is known that HSPs mediate antiapoptotic activity by interacting directly with caspases. The data presented suggest that induction of HSPs plays roles in cell protection exerted by many herbal supplements.

934 TEA TREE OIL ADMINISTERED ORALLY INDUCES SPECIFIC NEUROTOXICITY IN RATS.

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Tea Tree Oil (from Melaleuca alternifolia) is frequently used as a topical anti-irritant, and has been formulated in numerous consumer products, such as shampoos and toothpastes. Although toxicology data on Tea Tree Oil is limited, a number of studies have reported muscle weakness, ataxia and tremors in humans and other mammals following oral and topical administration. In this study, we examined neurotoxic effects of orally administered Tea Tree Oil. Specifically, we used a modified functional observational battery (FOB) to assess the development of specific neurotoxicity following Tea Tree Oil treatment. Groups of nulliparous female rats were administered increasing doses of Tea Tree Oil or sham control by oral gavage, and were observed at 1, 2, and 4 hours and then daily post-dose for signs of toxicity and specific neurotoxicity. At 72 hours post dose, all treated animals appeared to be lethargic, and exhibited markedly decreased overall activity patterns. Forelimbs and hindlimbs exhibited severe weakness and animals were unable to locomote normally; rats were dragging both forelimbs and hindlimbs and very few weight supporting steps were taken both in the home cage and testing arena. By day 4, all but one animal treated with ≤1.5g/kg appeared to have regained locomotor functions. In that one animal, the hindlimb functions were shown to be normal although forelimbs appeared to be completely paralyzed. The forelimbs remained immobile for the duration of the testing period. In groups administered with ≥1.6g/kg, the majority of animals displayed general ataxia, and forelimbs were affected to a much greater degree compared to the hindlimbs, remaining paralyzed for the duration of the testing period. These results suggest that Tea Tree Oil administered orally at doses greater than 1.5g/kg appears to induce neurotoxic lesions in pathways controlling limb movements.

935 13-WEEK FEEDING STUDY IN RATS FED GRAIN FROM ROUNDUP READY® EVENT NK603 CORN.

B. G. Hammond, B. R. Dudek, M. A. Nemeth and J. D. Astwood. *Monsanto Company, St. Louis, MO.*

As part of the safety assessment of food/feed derived from genetically modified crops, it is important to evaluate unintended consequences owing to the insertion of the transgene into the genome or expression of the resulting trait. In combination with other approaches such as compositional evaluations and nutritional assessments in farm animal feeding studies, a subchronic feeding study in rats may address such unintended effects. This study compared the responses of rats fed diets containing grain from Roundup Ready (RR) corn to rats fed (1)diets containing grain from near-isogenic control(C)(non-transgenic)corn and(2)a population of diets containing grain from commercial reference corn(RC)hybrids. Male and female CD IGS rats (20/sex/group) were fed one of the following diets for 13 weeks: 11 or 33 % (wt/wt)(RR)or(C)grain; 33% (RC) grain (6 commercial lines were used). There were a total of 10 groups/400 rats in the study. The diets that contained 11% RR or C grain were supplemented with 22% grain (non-transgenic commercial corn) to bring the total corn grain up to 33%, consistent with other diets. Laboratory rat feed contains corn grain at approximately 33%. Diets were balanced to be nutritionally equivalent. Animals were observed daily; body weights and food consumption were recorded at least weekly. After 5 and 13 weeks, blood and urine were collected from 10 animals/sex/group for clinical evaluations. After 13 weeks, all animals were sacrificed, necropsied and selected tissues weighed and examined microscopically. Body weights and food consumption were comparable for all groups. Clinical parameters were comparable across groups with few exceptions. The few differences were not considered biologically meaningful as they were either not dose related, or were not consistently different from both control groups. In conclusion, rats fed grain from Roundup Ready corn responded similarly to rats fed control grain. This conclusion was consistent with previous studies showing that RR corn grain is compositionally and nutritionally equivalent to traditional corn.

936 COMPARATIVE ACUTE AND COMBINATIVE TOXICITY OF AFLATOXIN B1 AND T-2 TOXIN IN RATS AND FISH.

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Aflatoxin B1 (AFB1) and T-2 toxin (T-2) are important food-borne biotoxins that have been implicated in human health and potential biological and chemical threats. In this study, acute and combinative toxicities of AFB1 and T-2 were tested

in F-344 rats and Mosquitofish (*Gambusia affinis*). Young male rats were randomly divided into five groups and were orally treated with 10.0, 4.64, 2.15, 1.0, and 0 mg/kg AFB1 or T-2 according to the Horn's method. The rats were observed for 7-days and lethality and toxic symptoms were recorded. Death of AFB1 treated rats appeared after 48 hrs, and death of T-2 treated rats, within 24 hrs. The LD50 for AFB1 was calculated as 2.71 mg/kg with the 95% of confident limit (CI) at 2.00-3.69 mg/kg and 3.71 mg/kg with 95% CI at 2.69-5.01mg/kg for T-2. Healthy fish (half male and female) were randomly assigned to 6 groups and treated with 4, 640, 2, 150, 1, 000, 464, 215, and 0 ppb of AFB1 or 2, 150, 1, 000, 464, 215, 100, and 0 ppb of T-2. The treated fish were observed for 5-days and death and toxic signs of fish were recorded. The LC50 for AFB1 was calculated to be 681 ppb with 95% CI at 420-800 ppb and LC50 for T-2 was 147.0 ppb with 95% CI at 95.1-227.0 ppb. Fish seem more sensitive to the acute toxicity than rats. Combinative studies used both biotoxins at doses of 1.0, 3/4, 1/2, 3/8, 1/4 LD50 or LC50 in both species. Synergistic and additive toxic effects were observed in rats and fish, respectively. These results will form the basis for our future study on combinative toxic and health effect of these biotoxins and their mixtures. This work was financially supported by the research contract DAAD13-00-C-0056 from Soldier and Biological Chemical Command (SBCCOM), US Army.

937 A NEW AETIOLOGY FOR RHABDOMYOLYSIS: *TRICHOLOMA* SPECIES INTOXICATION.

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Tricholoma equestre is a well-known mushroom considered to be edible and occurring worldwide. In France since 1992 it has been incriminated in 12 intoxications in humans. The intoxication has been seen after repetitive ingestion of these mushroom harvested in South West of France and is associated with 25% lethality. All subjects exhibited pure rhabdomyolysis without noticeable liver and kidney damage. The clinical pathology findings included large increases of circulating creatine kinase activity (CK) of up to 600 000 IU /L. Electromyography (EMG) and skeletal muscles histology confirmed the presence of rhabdomyolysis, without any neurological involvement. Using doses corresponding to mushroom quantities eaten by intoxicated patients, we have reproduced this intoxication in the mouse by gavage with *T. equestre*, the commercial mushroom *Pleurotus ostreatus* and p-phenylenediamine were taken as controls. Only in mice fed *T. equestre*, was circulating CK increased dose-dependently from 140 IU/L to 1600 IU/L. This was confirmed by histological images of mild disorganisation of muscle fibres in surviving mice. Some mice died (about 30%) following the highest dose. ASAT, ALAT, LDH and creatininemia were determined and did not show significant increase. Since all other aetiologies have been discarded in human cases of *T. equestre* intoxication and since this mushroom produces the similar tissue changes and death in animals we concluded that the consumption of large quantities of *T. equestre* may indeed be harmful and or lethal in humans.

938 *IN VITRO* SCREENING OF CRUDE AQUEOUS PREPARATIONS OF WHOLE FOODS FOR POTENTIAL TOXICANTS.

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The application of organic, conventional and molecular techniques may have the potential to alter the intrinsic levels of natural toxicants in crop plants. To maximize nutritional benefits and minimize the detoxification burden (metabolic cost) of ingesting crop foods, methods are needed to efficiently screen whole foods for unexpected changes in potential toxicity. We evaluated four *in vitro* mammalian toxicity screens utilizing crude, aqueous preparations of whole foods purchased from a local market. Thirty-seven whole foods were evaluated (0.05-10% vol. extract/vol. culture medium) in one or more of the following tests: (1) general toxicity (cytotoxicity, all 37 foods) and (2) genetic toxicity (chromosomal aberration test, 9 foods) both performed in Chinese hamster ovary cells; (3) developmental toxicity (limb bud micromass assay, 9 foods) using 11-day old CD-1 mouse embryos and (4) estrogenicity (MCF-7 cells transfected with a chimeric Gal4-estrogen receptor construct and a Gal4-regulated lucerifase reporter gene, 12 foods). Cytotoxicity levels ranged from <0.1% v/v (garlic) to non-cytotoxic (18 foods) at the maximal concentration tested (10% v/v). Five of the nine whole food preparations (soybeans, broccoli, garlic, snowpeas and corn) were clastogenic and two (soybeans and snowpeas) exhibited an ability to inhibit chrondrogenesis in the limb bud micromass assay. Four of nine preparations (soybeans, cumin, asparagus and bean-sprouts) produced positive estrogenic responses comparable to 17 beta-estradiol. Therefore, each of the tests showed some degree of response to these crude, whole foods extracts, and overall the 12 foods evaluated in two or more of the tests showed different patterns of response. These preliminary data indicate that screening for differences in potential toxicant profiles is possible with fast, relatively inexpensive *in vitro* tests.

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940 FOUR WEEK INHALATION STUDY WITH A MIXTURE OF DICHLOROETHYLENE AND PERFLUOROBUTYLENE IN RATS.

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A study was conducted to determine the subchronic toxicity of a mixture of trans-1, 2-dichloroethylene (trans-DCE, 70%), cis-1, 2-dichloroethylene (cis-DCE, 5%), and perfluorobutylethylene (PFBE, 25%). Groups of 20 male and 20 female rats were exposed by inhalation to 0, 400, 2000, or 8000 ppm of the mixture vapor for 6 hr/d, 5 d/wk for 20 exposures. Subgroups of 10 rats/sex/group were observed during a 1-month recovery period. Functional observational battery (FOB) and motor activity (MA) evaluations were conducted prior to initiation of exposures, during week 4, and at the end of recovery. Clinical pathology evaluations were conducted during week 4 and recovery. At the end of exposure, and at the end of recovery, tissues were collected for microscopic evaluation. Decreased body weights and weight gains occurred in 8000 ppm males and females. Decreased food consumption and/or efficiency occurred in 8000 ppm males. During exposure to 8000 ppm, rats exhibited tremors and ataxia. Tremors were also observed during exposure to 2000 ppm. Rats exposed to 2000 or 8000 ppm also had a diminished or no alerting response to a sharp sound stimulus during the daily exposures. No evidence of compromised neurological function were detected when the rats were evaluated upon return to their cages following exposure. There were no test substance-related effects on FOB parameters or MA conducted the day following the last exposure or during recovery. In addition, there were no compound-related changes in clinical chemistry parameters. There were no compound-related gross or microscopic morphological changes. The mixture produced neurological effects during the exposure period which where not observed previously with either trans-DCE or PFBE alone. Although cis-DCE has been previously reported to cause neurological effects during exposure, the effects were observed at concentrations of 13, 500 -15, 700 ppm. Therefore, the mixture appears to have an additive or synergistic effect in producing transient neurological effects during exposure.

941 FOUR-WEEK INHALATION TOXICITY OF METHYLGLUTARONITRILE IN RATS.

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Methylglutaronitrile (MGN) is a high-boiling (263 °C) solvent/intermediate used in the fiber industry. Twenty male rats per group were exposed nose-only to approximately 5, 25, or 200 mg/m3 of MGN for 6 hours/day, 5 days/week over a 4 week period. Only a small amount of aerosol (<0.2 mg/m3) was present at the two lowest concentrations, the rest was vapor; however, 160 mg/m3 of aerosol was present at the highest concentration (MMAD 3.8 µm) along with 40 mg/m3 of vapor. Ten rats/group were sacrificed one day after the final exposure and the remaining rats after a four-week recovery period. Rats were given clinical, neurobehavioral, and pathologic examinations. No effects were observed in clinical observations during the exposure period, but body-weight gains were depressed in the 200 mg/m3 group. The 200 mg/m3 group showed minimal decreases in RBC, HGB, and HCT values accompanied by increases in reticulocytes followed by decreased reticulocytes at the end of the recovery period. There were no other effects observed in clinical, neurobehavioral, or pathologic evaluations in the study. During the 4-week recovery, weights in the high level group returned to normal and there were no other clinical or pathological effects observed in any groups. Based on these results, MGN was considered to have low toxicity in rats in repeated inhalation exposures.

942 FOUR-WEEK INHALATION TOXICITY OF N-VINYL CARBAZOLE IN RATS.

D. P. Kelly, G. T. Makovec and G. S. Ladics. *Haskell Laboratory, DuPont Co., Newark, DE.*

n-Vinyl carbazole (nVC) is a component in photo film. Fifteen male and 15 female rats per group were exposed nose-only to condensation aerosol/vapor concentrations of approximately 0, 2, 9.5, or 44 mg/m3 of n-vinylcarbazole (nVC) for 6 hours/day, 5 days/week over a 4 week period. Rats were exposed to mainly aerosol along with 0.4, 0.6 and 0.4 mg/m3 of vapor, respectively. Ten rats/group were sacrificed one day after the final exposure and the remaining rats after a four-week recovery period. No effects were observed in clinical observations or body weight de-

terminations during the exposure phase or recovery phase of the study. However, pathological effects were observed at all concentrations in nVC exposed rats of both sexes. The effects were confined to the upper respiratory tract and consisted of degeneration/necrosis of olfactory epithelium, mucoid depletion of goblet cells, and squamous metaplasia and hyperplasia of respiratory epithelium in the anterior nasal passages and squamous metaplasia of the larynx. At the end of the four-week recovery period, the depletion of goblet cells in noses of female rats was no longer present, and was of decreased severity in the male rats, however, the olfactory epithelial degeneration/necrosis was still present in both sexes. Further work is underway to determine a no-effect level for nVC exposure in rats.

943 DIMETHYL GLUTARATE (DMG), DIMETHYL SUCCINATE (DMS), AND DIMETHYL ADIPATE (DMA): 90-DAY INHALATION TOXICITY STUDY IN RATS.

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Groups of male and female rats were exposed *via* inhalation to 0, 10, 50, or 400 mg/m^3 DMG, 400 mg/m^3 DMS, or 400 mg/m^3 DMA for 6 hrs/day, 5 days/wk over a 90-day period. The exposure period was followed by a 1-month recovery period. Rats were evaluated for clinical signs and growth throughout the study. Samples for hepatic, lung, and nasal cell proliferation (CP) were collected from rats approximately 2 weeks and 90 days after study initiation. Clinical pathology, anatomical pathology, male reproductive endpoints, neurobehavioral assessments, neuropathological evaluation, estrous cycle, and hormonal analyses were conducted during the study. No compound-related effects were observed on mortality, clinical signs, clinical pathology, neurobehavioral endpoints, neuropathology, sperm motility or morphology, or estrous cycle. Male rats exposed to 400 mg/m^3 DMG had lower mean body weights and mean body weight gains during the study. Compound-related focal and minimal effects were observed in the noses of rats exposed to 400 mg/m^3 of DMG, DMS, or DMA for 90 days. CP in the noses of rats exposed to 400 mg/m^3 of DMG, DMS, or DMA was increased compared to controls during the study. In male rats exposed to DMG, serum testosterone concentrations were statistically significantly decreased at concentrations of 50 and 400 mg/m^3. Serum luteinizing hormone concentrations were decreased in a dose-dependent manner and were statistically significantly decreased at 400 mg/m^3. In female rats, DMS caused a statistically significant decrease in serum estradiol concentrations. There was an increase in epididymal sperm counts following exposure to DMG and the number of sperm per cauda and per gram cauda epididymis was significantly increased at 50 and 400 mg/m^3. In male rats exposed to DMS, epididymal sperm counts were significantly increased. In this study, the no-observed-effect level (NOEL) for repeated exposure to DMG was 10 mg/m3. NOELs for DMS and DMA were not established. Sponsored by the Dibasic Esters Group, Washington DC.

944 ROLE OF SENSORY NERVES IN THE AIRWAY AND CARDIAC RESPONSES TO SULFUR DIOXIDE (SO_2) IN A GUINEA PIG MODEL.

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Sulfur Dioxide (SO_2) is a common component of polluted urban air. Exposure to SO_2 has been shown to significantly alter airway compliance and resistance. Recently, high concentrations of SO_2 during air pollution episodes have been associated with heart rate elevation. Using non-invasive methodology, our laboratory has previously shown that exposure to SO_2 produces a concentration dependent increase in airway resistance and a significant decrease in breathing frequency. The purpose of our current study is to: 1) determine if exposure to SO_2 produces simultaneous changes in airway and cardiac function and, 2) ascertain the role of sensory nerves in these responses. To this end, guinea pigs were surgically implanted with radiotelemeters capable of transmitting ECG, heart rate and core body temperature data in 1-minute intervals. Animals were then exposed to either 0 or 100 ppm SO_2 in the head-space of a double-chamber plethysmograph. Airway and cardiac function were monitored continuously for a 10-minute baseline period and the 60-minute exposure period. Exposure to SO_2 produced a significant decrease in heart rate during the second half of the exposure period ($p<0.05$). To determine the role of sensory nerves in these responses, animals were pretreated with the neurotoxin, capsaicin (total dose 125 mg/kg sc). Pretreatment with capsaicin significantly diminished the SO_2-induced increase in SRAW and decrease in breathing frequency ($p<0.05$). In addition, capsaicin pretreatment attenuated the SO_2-induced decrease in heart during the second half of the exposure period ($p<0.05$). In summary, these results suggest that exposure to SO_2 produces simultaneous changes in both airway and cardiac function and that stimulation of sensory nerves play a role in both of these responses. (Funded by the NCSU/EPA Cooperative Training Program CT826512010.) (This abstract does not reflect EPA policy.)

945 EFFECTS OF WELDING FUMES ON LUNG INJURY AND INFLAMMATION: THE POSSIBLE ROLE OF FREE RADICAL PRODUCTION.

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The goals of this study were to examine lung damage and inflammation as well as free radical production caused by welding fumes of different composition. Fume was collected during flux-covered manual metal arc welding using a stainless steel consumable electrode (MMA-SS) and gas metal arc welding using a mild steel electrode (GMA-MS) or a stainless steel electrode (GMA-SS). Metal composition analysis by energy dispersive spectroscopy revealed that only MMA-SS was highly soluble, containing both soluble Cr and Mn, while GMA-MS and GMA-SS were relatively insoluble. Using electron spin resonance, hydroxyl radicals were observed in a suspension of MMA-SS fume containing hydrogen peroxide. Both GMA fumes showed no evidence of free radical production. To examine the effects of the fumes on lung damage and inflammation, male Sprague-Dawley rats were intratracheally instilled with either a welding fume suspension at 2 mg/rat or the saline vehicle. On day 3, the right lung was assayed for lipid peroxidation (LPO) products while the left lobes were subjected to bronchoalveolar lavage (BAL). LPO products were elevated in the lungs following MMA-SS treatment only, implicating possible oxidative damage. All fumes caused increases in BAL cell number, including elevated macrophage and neutrophil recovery. However, only MMA-SS led to an increased recovery of eosinophils, indicating a possible immune reaction. MMA-SS treatment caused an elevation of albumin in the first BAL fraction, indicating damage to the alveolar-capillary barrier, while all fumes produced an increase in lactate dehydrogenase activity in that fraction. Total protein in the first BAL fraction was most elevated following MMA-SS treatment. These findings indicate that different welding fumes caused varied responses in the lungs of rats, and that these responses may be correlated to the soluble metal composition of the fume and the ability of the fume to produce free radicals.

946 TEMPORAL CHANGES IN BAL ELECTROLYTE LEVELS IN MICE AFTER EXPOSURE TO AN EDEMAGENIC GAS.

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Detection of acute lung injury is important if therapeutic medical countermeasures are to be used to reduce toxicity in a timely manner. Indicators of injury may aid in the eventual treatment course and enhance the odds of a positive outcome following a toxic exposure. This study was designed to investigate the effects of a toxic exposure to the industrial irritant gas phosgene on the electrolyte levels in bronchoalveolar lavage (BAL) fluid. Phosgene (CG) is a well- known chemical intermediate capable of producing life-threatening pulmonary edema within hours after exposure. Four groups of 40 each CD-1 male mice were exposed whole-body to either air (A) or a concentration x time (c x t) amount of 32 mg/m3 (8 ppm) CG for 20 min (640 mgxmin/m3). BAL from A- or CG-exposed mice was taken at 1, 4, 8, 12, 24, 48, and 72 h postexposure. After euthanasia, the trachea was excised, and 800 ul of saline was instilled into the lungs. The lungs were washed 5x. Eighty ul of BAL was placed in a cartridge and inserted into a clinical I-STAT analyzer. Na+, K+, Cl-, and ionized Ca++ were analyzed within 2 minutes of insertion. The left lung was removed to determine wet weight (WW), an indicator of pulmonary edema. Temporal changes in BAL Na+ and Cl- in CG mice were not statistically different from those in the A mice. Both Ca++ and K+ were significantly higher than in the A mice over 72 h, $p<.03$ and $p<.001$, respectively. Significant changes in BAL Ca++ and K+ occurred as early as 4 h postexposure in CG, $p<.05$, *versus* A mice. CG caused a time dependent significant increase in WW from 4-12 h, $p<.05$, compared with A mice. BAL Ca++ at 1 hr in CG-exposed was 60% higher than in the time-point matched A mice. These data indicate that measuring BAL electrolyte levels may serve as an alternate indicator of lung injury since both Ca++ and K+ follow temporal increases in tissue damage. In addition, knowledge of these levels may indicate how rational therapeutic intervention could be most beneficial.

947 OZONE EXPOSURE ENHANCES ANTIGEN-PRESENTING ACTIVITY ON BRONCHOALVEOLAR LAVAGE CELLS CONCENTRATION DEPENDENTLY IN RATS.

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Previous studies have shown that O_3 exposure aggravates allergy like diseases such as asthma and rhinitis. Our previous studies have shown that expression of cell-surface molecules associated with antigen presentation on bronchoalveolar lavage cells

(BAL cells) is increased by exposure to O_3 in rats. The present study designed to investigate concentration dependencies of the effect of O_3 on the expression of cell-surface molecules associated with antigen presentation and on accessory activity of BAL cells. Male Wistar rats were exposed to 0.3, 0.56, 1 ppm O_3 or filtered air for 3 days. Expression of cell-surface molecules (Ia, B7.1, B7.2, and CD11b/c) was measured by flow cytometry. Accessory activity of BAL cells was assessed by the allogeneic mixed lymphocyte reaction (MLR) and ovalbumin (OVA)- specific antigen-presenting activity. The numbers of Ia^+, $B7.2^+$, $CD11b/c^+$, $Ia^+/B7.2^+$, and $Ia^+/CD11b/c^+$ BAL cells were increased by O_3 exposure concentration dependently. The accessory activity of BAL cells measured by MLR and OVA-specific antigen-presenting activity was also enhanced by O_3 exposure concentration dependent manner. These results suggest that the enhancement of antigen-presenting activity of BAL cells is caused by the increasing of expression of cell-surface molecules associated with antigen presentation by O_3 exposure. This enhancement may trigger the subsequent immune response that could make worse allergic asthma and rhinitis.

948 ULTRASTRUCTURAL CHANGES IN THE AIRWAYS OF RATS INHALING BUTTER FLAVORING VAPORS.

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A cluster of eight cases of fixed airway obstruction occurred in former workers in a microwave popcorn plant. As part of a request to NIOSH to find the cause, extensive medical and environmental measurements were made at the plant. High levels of organic gases and decreased pulmonary function among workers were associated with butter flavoring in use at the plant but other exposures existed. To assess the biological effects of the flavoring, we exposed rats by inhalation for 6 hours to butter flavoring vapors. Qualitative GC-MS analysis yielded a variety of organic gases including diacetyl and acetoin. Average diacetyl concentration in the exposure chamber was about 350 ppm. By transmission electron microscopy (TEM), the principal finding in the mainstem bronchus of exposed rats was airway epithelial necrosis. Unusual features of the epithelial alterations included rarefaction of basilar cytoplasm and relative preservation of cilia. Denuded basement membrane was frequently observed although a thin fragment of basilar cytoplasm of respiratory epithelium remained attached in some foci. Damage in the mainstem bronchus extended beneath the basement membrane into the lamina propria where edematous changes were characterized by disorganization and separation of collagen fibers and fibrils. Necrotizing changes were less frequent in smaller airways. By scanning electron microscopy (SEM), ulceration of bronchiolar epithelium tended to be localized at bronchiolar bifurcations. No evidence of alveolar injury was evident in TEM or SEM sections. These findings document that artificial butter flavoring, while considered safe to consume at concentrations present in food, produces vapors capable of inducing severe airway injury in laboratory animals when inhaled at high concentrations similar to peak exposures in the workplace.

949 SPECIES DIFFERENCE IN ACUTE RESPIRATORY RESPONSES TO VAPOR AND AEROSOL IRRITANTS BETWEEN RATS AND MICE.

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This study was to compare acute respiratory responses upon exposure to a vapor (cumene) or an aerosol (vanadium pentoxide, V_2O_5) irritant between young male F344 rats and SW mice. Animals (4/species/chemical/dose/timepoint) were exposed to either chemical and their tidal volume (TV), respiratory rate (RR), and minute volume (MV) were measured. For cumene exposure, respiratory function was measured for 10-min preexposure and during 30-min nose-only inhalation exposure to 100 or 300 ppm cumene. For V_2O_5 exposure, animals were exposed *via* intratracheal instillation (0, 0.63, or 6.3 mg V/kg in saline) and respiratory function was measured 1 and 3 days post dosing. For V_2O_5-instilled mice, bronchoalveolar lavage (BAL) was performed, and data were compared with those from V_2O_5-instilled rats under the same study design (Lee et al., 2000). Cumene exposure at both concentrations significantly depressed TV and RR of mice, resulting in 39-41% reduction in MV compared to preexposure baseline. In rats, reduced respiratory function was observed only at 300 ppm (38% reduction in MV). For V_2O_5-instilled mice, respiration became rapid (RR ↑–) and shallow (TV ↓), which was most severe with the high-dose group on day 1 (MV reduced by 37% compared to saline-instilled controls). Signs of pulmonary inflammation (increases in LDH, NAG, protein, and neutrophil count in BAL fluid) were also observed in V_2O_5-instilled mice. For V_2O_5-instilled rats, rapid and shallow breathing was most severe in high-dose group on day 3 (MV reduced by 43%). Therefore, although acute respiratory responses between rats and mice varied depending on the exposure concentration and time, mice appeared to be more sensitive in terms of modifying respiratory functions or inducing inflammatory responses upon single exposure to these respiratory irritants.

950 INITIAL CONCENTRATIONS AND DISAPPEARANCE OF SELECTED MALODOROUS COMPOUNDS FROM FRESH AND AGED DAIRY MANURES.

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Malodorous compounds from livestock manures are offensive and can cause health hazards to humans and cattle. Changes of manure storage and handling methods may prevent the formation and/or enhance the degradation of odors. This study was to quantify volatile fatty acids (VFAs), phenol, cresols, indoles and NH_3 in and emitted from fresh and 12-day-aged manure. Loss of these compounds was monitored during pilot-scale composting in 205 L vessels either aerated continuously (AC) with high (2.3 kg/hr)/low (0.8 kg/hr) air flow controlled by thermostats or intermittently (AI) on a 5 min high air flow 55 min off cycle. Manures were mixed with sawdust (3:1, w/w). Six replicates were conducted on each treatment combination, with 16 day trials. Emissions from the composting masses were measured. FID/GC was used to analyze VFAs in condensates and pH 2 water extracts of solids. Ether extracts of phenolics and indolics were quantified by GC/MS. Mean NH_3 emissions from fresh and aged manure AI were 50 and 60 g. Fresh and aged manure AC emitted 121 and 110 g NH_3. NH_3 peaked early and decreased to undetectable by day 17. Aged, compared to fresh manure, contained greater varieties and amounts of VFAs, phenols and indoles with the most offensive odors. Fresh manure contained acetate, propionate, isobutyrate, isovalerate, phenol, p-cresol, and indole (5000, 500, 40, 70, 35, 200 and 5 μg/g). Aged manure contained acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, phenol, p-cresol, indole and skatole (9000, 2700, 200, 2500, 2800, 350, 50, 350, 10 and 30 μg/g). By day 8, trace quantities of acetate remained in fresh manure, while acetate, propionate and butyrate (900, 120 and 130 μg/g) were in aged manure. Acetate, propionate and butyrate co-distilled with water being highest from aged manure. Bioprocesses associated with AC and AI influenced the loss of certain chemicals. Avoiding anaerobic aging of dairy manure was important in reducing concentrations of the chemicals studied.

951 WHOLE BODY EXPOSURE SYSTEM FOR ADMINISTERING NITRIC OXIDE (KINOX®) BY INHALATION TO NEONATE RATS.

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A whole body exposure system has been designed in order to expose neonate rats to controlled concentrations of nitric oxide for inhalation toxicity studies and to prevent levels of the oxidation product NO_2 rising above 1 ppm for an atmosphere containing 100 ppm NO. The exposure chambers comprise a polycarbonate nesting box with a perforated floor and a pyramidal lid incorporating a gas mixing chamber. Each exposure chamber accommodates a dam plus litter. A nitric oxide/nitrogen mixture of known concentration, oxygen and clean air are mixed and pass into the chamber *via* a duct in the chamber lid. The atmosphere is exhausted through the perforated floor. Gas flow rates are set to ensure a minimum residence time in the chamber to prevent NO_2 build up while maintaining temperature and relative humidity in the chamber conducive for exposure of animals. The NO/N_2 mixture, oxygen and airflow rates are controlled, monitored and recorded throughout exposure. An automated sampling and data capture system monitors the chamber concentrations of NO and NO_2 in real time. An inhalation toxicity study was conducted where neonate rats were exposed for 6 hours a day with dams from Days 2–10 *post-partum* and litters alone from Days 11-29 *post-partum*, target levels of NO were 10, 30 and 100 ppm. Mean achieved concentrations of NO were 10.2, 30.3 and 100.2 ppm. Concentrations of NO_2 were 0.0, 0.2 and 1.1 ppm. In conclusion, the study results demonstrate that the system is capable of excellent control of NO concentration and can maintain the required low levels of NO_2.

952 ALDEHYDE DEHYDROGENASE (ALDH2) POLYMORPHISM AND THE PULMONARY EFFECTS ASSOCIATED WITH EXPOSURE TO ETHANOL VAPORS IN THE RAT.

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Addition of ethanol to gasoline aiming at reducing the greenhouse effect is likely to increase atmospheric emissions of ethanol and acetaldehyde. By converting acetaldehyde to acetate ALDH2 plays a major role in the metabolism and toxicity of EtOH. A significant polymorphism exists in both humans and rats. In the latter, a

point mutation gives rise to a slow metabolizing enzyme. This mutation is present on the ALDH2R allele but is absent on the ALDH2Q allele, with the ALDH2R allele expressing an incomplete dominance. Our hypothesis is that atmospheric ethanol may cause pulmonary toxicity and that individuals expressing the ALDH2R allele could be at greater risk. ALDH2 polymorphism was determined by PCR amplification of the polymorphic site followed by a digestion with DdeI enzyme. Characterization of the various genotypes in a group of Sprague-Dawley rats (Charles River Canada) (N=115) gave the following distribution: 2R/2R (17%), 2Q/2R (53%), 2Q/2Q (30%). Pulmonary ALDH2Q/2Q isozyme showed greater *in vitro* metabolic activity, assessed by NADH formation, than ALDH2R/2R isozymes. Inhalation exposure experiments (500 ppm, 6h) showed that EtOH is rapidly excreted from blood., i.e. around 94 % of the dose is excreted in 60 min for female rats. Blood levels of acetaldehyde could not be detected in these rats suggesting its binding to proteins. Intratracheal instillation of ^{14}C-EtOH in female rats showed that after a 3 h period, ethanol-derived radiolabeled material binds to pulmonary proteins. Ongoing studies are aimed at determining if differences in amounts bound exist between ALDH2Q/2Q and ALDH2R/2R genotypes. Similarities between acetaldehyde and formaldehyde binding to proteins suggest that EtOH vapors may lead to pulmonary toxicity. Overall, this work may improve the health risk assessment associated with the addition of ethanol to gasoline. (Supported by the Toxic Substances Research Initiative)

953 THE EFFECT OF COMBUSTION OF MOSQUITO COILS ON CHILDREN'S RESPIRATORY HEALTH.

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We report a significant impact on the respiratory health of children due to combustion of mosquito coils. Two groups of healthy Malaysian schoolchildren, ages 7-11, from 3 rural schools were chosen based on exposure to indoor air pollution. One group of 125 children came from homes in which there was cigarette smoking, home cooking twice or more daily, and the burning of insecticide-impregnated mosquito coils at night to protect children from mosquitos. The control group of 156 children was negative for all of these characteristics. Pulmonary function studies showed that the FVC and FEV1 in girls and boys were each more than 10% lower in the exposed groups ($p<.001$ for each). Respiratory symptoms were much higher in the exposed group; e.g., chronic cough was observed in 25/125 children in the exposed group vs 2/156 in the exposed group ($p<.001$). Further analysis showed that the pulmonary function and symptom effects for the most part could be attributed to the mosquito coils. Studies of combustion products from mosquito coils from Malaysia and China revealed high levels of fine particulates and aldehydes. Coupling the observed emission rates with the range of expected room sizes and air exchange rates suggest that night time exposures to PM2.5 would range from 129 to 4, 340 ug/m3 (US annual std 15 ug/m3); to formaldehyde 3.3 to 131.3 ug/m3; and to acrolein 0.10 to 5.8 ug/m3 (EPA chronic exposure effect level for formaldehyde 3 ug/m3 and for acrolein 0.06 ug/m3). It is conceivable that at least 100 million children in Asia, Africa and Latin America are exposed indoors to burning mosquito coils.

954 RAT SUBCHRONIC INHALATION STUDY OF SMOKE FROM CIGARETTES CONTAINING FLUE-CURED TOBACCO CURED EITHER BY DIRECT-FIRED OR HEAT EXCHANGER CURING PROCESSES.

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A subchronic nose-only inhalation study compared the effects of smoke from a cigarette consisting of 100% flue-cured tobacco cured by a direct-fired process to that of a cigarette consisting of 100% flue-cured tobacco cured by a heat exchanger process. The heat exchanger process applies indirect heat to the tobacco *via* a heat exchanger device. Sprague-Dawley male (M) and female (F) rats were exposed for 1 hour a day, 5 days per week, for 13 weeks to mainstream smoke at 0, 0.06, 0.20, or 0.80 mg/liter of air. Neither cigarette produced adverse changes in clinical signs, body and organ weights, serum chemistry, or hematology. Carboxyhemoglobin and serum nicotine increased with both cigarettes in an exposure-dependent manner. Smoke related histologic changes were nasal goblet cell hypertrophy, nasal chronic/active inflammation and epithelial hyperplasia, chronic/active inflammation of the larynx, squamous metaplasia and acute inflammation of the ventral larynx, tracheal epithelial hyperplasia and increased goblet cells, increased goblet cells of the lung, and increased non-pigmented and brown/gold pigmented lung macrophages. When histologic changes resulting from exposure to smoke from the two types of cigarettes were compared, the only observed difference was increased epithelial hyperplasia of the anterior nasal cavity in males in the high exposure group for the heat exchanger cigarette. Since only one difference was noted among all of the observed smoke related histologic changes, these findings indicate that the biological activity of smoke from cigarettes of flue-cured tobacco cured by a direct-fired or a heat exchanger curing process is not different under the conditions of the study.

955 CHRONIC EFFECTS OF INHALATION OF ROOM-AGED SIDESTREAM SMOKE AND DIESEL ENGINE EXHAUST IN THE RAT RESPIRATORY TRACT.

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Environmental tobacco smoke (ETS) and Diesel engine exhaust (DEE) are widespread environmental combustion aerosols found at similar concentrations. Similar relative risks for lung cancer have been reported for both. The purpose of this investigation was to determine the carcinogenic potency of room-aged sidestream smoke (RASS), as a surrogate for ETS, and DEE at toxicologically relevant concentrations that bear a realistic relationship to human exposure. Male and female Wistar rats were nose-only exposed to fresh air and to RASS and DEE at 3 and 10 mg/m^3 for 6 h/day, 7 d/week for 24 months followed by a 6-month postinhalation period. To increase the sensitivity for the detection of lung tumors, 0.5 mm step serial sections were utilized. The only findings in the nose, larynx, and trachea were hyperplasia and metaplasia. In the lung a few RASS-related non-neoplastic findings (goblet cell hyperplasia, pigmented alveolar macrophages) were observed. There was a dose-dependent increase in the severity of DEE-associated non-neoplastic changes, which comprised alveolar epithelium hyperplasia, bronchoalveolar metaplasia, squamous metaplasia, alveolar, interstitial and pleural particle-filled macrophages/particulate matter, chronic active inflammation, and septal fibrosis. The incidence of lung tumors (102 rats/group) in the RASS-exposed groups was 5% each (adenomas and one adenocarcinoma) and not statistically significantly different from sham (2%). In DEE-exposed groups lung tumors first appeared between 12 and 24 months. The incidence of tumors increased with dose (up to 50%) and comprised benign cystic tumors, adenomas, adenocarcinomas, and squamous cell carcinomas. This spectrum of neoplastic and non-neoplastic findings in rats exposed to DEE is consistent with the literature. No alveolar hyperplasia was seen at any time point in the RASS groups, which suggests that the tumors in the RASS groups were spontaneous in nature. INBIFO is a Philip Morris research laboratory.

956 NEUROKININ RECEPTOR-1 GENE EXPRESSION IN MICE SUBJECTED TO SIDESTREAM CIGARETTE SMOKE.

S. J. Wang, N. N. Sun and M. L. Witten. *Pediatrics, University of Arizona, Tucson, AZ.*

There are unmyelinated afferent nerves (C-fibers) that serve as an important sensory system in detecting the onset of pathophysiological changes in the lungs. To investigate whether the gene activation of neurokinin receptor-1 (NK-1) following sidestream cigarette smoke (SSCS) exposure, the elderly C57BL/6 mice were randomly assigned to 5 groups (10/group) in a study of the dose-effect relationship. The mice of each group were exposed to 0 (room air), 2, 4, 8, and 16 standard research cigarettes (1R4, University of Kentucky)/day, respectively, for 7 days through a nose-only exposure chamber (IN-Toxicology). The expression of NK-1 mRNA in the lung, heart, liver, kidney, and spleen tissues were detected by RT-PCR techniques and normalized against GAPDH expression. The mice subjected to lower SSCS exposure had a downregulation of NK-1 mRNA expression in heart, but an upregulation in liver and kidney when compared to control rats. However, less or more induction for the NK-1 gene was observed following the higher dosages of SSCS exposure when compared to the lower dose of SSCS exposure in these tissues. This finding suggests that there are the paracrine and/or autocrine signaling mechanisms through receptor-ligand interactions. No alteration of NK-1 gene expression was observed in the lungs and spleen tissues in this study. In conclusion, activation of NK-1 gene expression may contribute to passive cigarette smoke-induced lung responses, which may be of primary importance in shifting neurogenic inflammatory responses from their physiological protective/defensive functions to detrimental pathophysiological roles. (Supported by ADCRC No. 9925).

957 REVERSES OF β-CHEMOKINE RECEPTOR GENE EXPRESSION IN C57BL/6 MICE EXPOSED BY SIDESREAM CIGARETTE SMOKE.

N. N. Sun, S. Wang and M. L. Witten. *Pedatrics, University of Arizona, Tucson, AZ.*

Chemokines have recently been shown to play an important role in the activation and directional migration of inflammatory cells to sites of tissue injury. Their action requires the expression of their complementary chemokine receptors by their target

cells. However, the possible action of β-chemokine receptors (CCR) genes in the pathogenesis of environmental tobacco smoke has not been established. To examine if passive cigarette smoke can activate CCR gene expression, the lung, heart, liver, kidney, and spleen tissues were isolated from C57BL/6 mice, which are daily exposed to sidestream cigarette smoke (SSCS) from 0 (room air), 2, 4, 8, and 16 standard research cigarettes (1R4, University of Kentucky), respectively, for 7 days. The expression of CCR-1 to CCR-5 genes in these tissues were detected by RT multiplex PCR techniques and normalized against GAPDH expression. CCR-1 to CCR-5 mRNA levels of mice exposed to lower SSCS concentrations basically present a decreased trend in the lung, heart, and kidney tissues, but an increased trend in liver and spleen. However, significant reverses for these CCR gene expressions in heart and kidney tissues were observed following the higher dosages of SSCS exposure, suggesting there are the paracrine and/or autocrine signaling mechanisms through receptor-ligand interactions. The changes in CCR gene expressions in the tissue suggests the b-chemokine receptor and its ligand, such as Eotaxin-1, Eotaxin-2, Eotaxin-3, RANTES, MCP-2, 3, 4, and MIP-5, may play an important role in the recruitment of inflammatory cells following SSCS exposure. (Supported by ADCRC No. 9925).

957A OZONE TOXICITY IS ASSOCIATED WITH UPREGULATION OF PHOSPHATIDYLINOSITOL 3-KINASE (PI3K) AND PROTEIN KINASE B (PKB) IN LUNG MACROPHAGES. ROLE OF CAVEOLIN-1.

L. Fakhrzadeh, K. Lee, S. G. Rickatts, C. R. Gardner, V. S. Sunil, J. D. Laskin and D. L. Laskin. *Joint Graduate Program in Toxicology, Rutgers University and UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.*

Exposure of mice to toxic levels of ozone (O_3) causes type I epithelial cell damage and type II cell proliferation. This is associated with increased expression of inducible nitric oxide synthase (NOSII) and production of reactive nitrogen intermediates by alveolar macrophages (AM), which have been implicated in tissue injury. In the present studies we analyzed mechanisms regulating NOSII activity in AM after O_3. The promoter region of the NOSII gene is controlled by several transcription factors including NF-KB. O_3 inhalation (0.8 ppm, 3 h) caused a time-dependent induction of NF-KB nuclear binding activity in AM. The findings that AM isolated from knockout mice lacking the p50 subunit of NF-KB did not generate nitric oxide, and that the mice were protected from toxicity demonstrate that signaling through NF-KB is important in O_3 induced lung injury. Activation of NF-KB requires phosphorylation of IKB kinase, as well as NF-KB subunit proteins. O_3 inhalation resulted in a time dependent induction of phosphatidylinositol 3'-kinase (PI3K) and its downstream target Akt/protein kinase B (PKB) in AM. Moreover, treatment of the cells with the PI3K inhibitors, wortmanin and LY294002, blocked nitric oxide production. This was correlated with reduced NF-KB nuclear binding activity in the cells. PI3K activity is controlled by caveolins, a family of highly conserved integral membrane proteins. We found that caveolin-1 (Cav-1) was constitutively expressed in AM. O_3 exposure caused a marked reduction in Cav-1 in AM, which was evident immediately following exposure and persisted for 6 h. Changes in expression of Cav-1 were inversely correlated with expression of PI3K and PKB in AM. Taken together, these data suggest that Cav-1 may play a role in PI3K and PKB signaling and NF-KB activation leading to nitric oxide production following O_3 inhalation. Supported by NIH grants ES04738 and ES06897.

958 A METABONOMIC APPROACH TO STUDY THE ONSET OF NEUROTOXICITY USING 3-NITROPROPIONIC ACID, A MODEL FOR HUNTINGTON'S DISEASE.

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The model toxin 3-nitropropionic acid (3-NP) replicates, in animals, most of the pathological features, cognitive and motor dysfunctions that are observed in humans with Huntington's Disease [1]. We have used a metabonomic approach to study the onset of toxicity in male Sprague-Dawley rats (n=10 per group) following daily dosing with 3-NP (5, 7.5 mg/kg/day) over a 14 day period. Urine samples were collected throughout the study for NMR spectroscopic analysis and histological examinations were carried out on all animals at study termination. Rats treated with 3-NP exhibited weight loss, reduced food and water consumption and a degree of functional impairment. Although the animals exhibited symptoms ranging from ataxia to right hind-limb paralysis in all dose groups, histology failed to show the expected neuronal loss in brain tissue. Clinical findings were accompanied by mild to moderate decreases in urinary pH from 8hrs onwards. However, NMR analysis demonstrated a marked increase in the excretion of urinary succinate immediately post-dose until the end of the study. Multivariate statistical analysis using principal components analysis (PCA) confirmed an increase in succinate as the predominant biochemical change and demonstrated a Dose-Response relationship in the separation of 3-NP dosed animals from controls. The increase in urinary succinate excretion following administration of 3-NP has not been reported previously, but is consistent with the observation that 3-NP is an irreversible inhibitor of succinate dehydrogenase [2]. This result suggests a gross impairment of energy metabolism in the TCA cycle, which could be the cause of neuronal cell death. The observation of succinic aciduria in the absence of the expected brain pathology demonstrates the utility of NMR spectroscopy and the metabonomic approach as an adjunct to standard toxicological techniques, particularly during the onset of toxicity. 1. Acta Medica 43, 9–13 (2000) 2. Journal of Biological Chemistry 254, 5161-5167 (1979)

959 EFFECT OF DEXAMETHASONE ON THE METABONOMICS PROFILE ASSOCIATED WITH PHOSPHODIESTERASE (PDE) INHIBITOR-INDUCED MESENTERIC VASCULAR LESIONS IN RATS.

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Metabonomics is a novel technology proposed as a predictive tool for screening novel compounds and for identification of biomarkers of toxicity in biological fluids. Utilizing this approach, we previously identified urinary spectral patterns in rat characteristic for vascular lesions induced by suprapharmacological doses of the type IV PDE inhibitor CI-1018. Whether the observed changes in metabonomics profile were related to the mechanism of toxicity or were instead a measure of the subsequent inflammatory response was uncertain. In the present study, dexamethasone (DEX) was used to examine the relationship between the inflammatory process and the metabonomics profile induced by CI-1018. Six female Wistar rats were given 5 daily doses of DEX (1 mg/kg IP), 4 daily doses of CI-1018 (750 mg/kg PO), or given a predose of DEX followed by 4 daily doses of DEX plus CI-1018. Twenty-four-hour urine samples were collected from each rat pretest and daily for the duration of the study for metabonomic analyses. All rats dosed with CI-1018 alone had marked mesenteric and/or mild hepatic vascular lesions characterized by medial necrosis, hemorrhage and/or edema accompanied by perivascular mixed inflammatory cell infiltrates. Minimal arteriopathy without concurrent inflammatory infiltration was noted in 5 of 6 rats given both dexamethasone and CI-1018. Principle component analysis performed on urine samples revealed an obvious pattern of separation between samples from control rats *versus* those from CI-1018-treated rats with marked lesions and rats given DEX plus CI-1018 with minimal lesions and no inflammatory cell infiltration. Pattern separation was not evident between the two CI-1018 treatment groups. The separation from controls, observed in rats with minimal lesions without inflammatory infiltrates, suggests that the observed shift in spectral pattern is independent of the inflammatory process associated with vascular injury induced by PDE inhibition.

960 HEPATIC OXIDATIVE STRESS RESPONSE TO LAZABEMIDE.

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Tempium® (Lazabemide: Ro 19-6327) is a potent, highly selective and reversible inhibitor of MAO-B with clinical advantages over current therapies for the treatment of Alzheimer's disease. During the pre-clinical safety program an increase in rat liver enzymes with some similarity to clinical findings was observed. In a limited number of patients, transient increases in ALT and GGT and less frequently AST, have been recorded between weeks 6 to 12 of lazabemide treatment (100 mg bid). In one patient, however, plasma enzymes continued to increase after withdrawal of treatment but eventually declined to reference levels. Based upon this data and the pre-clinical findings the transient and reversible increase in liver-related enzymes appears to affect patients and experimental rats alike. Further investigative work was initiated to address the mechanism of this hepatic response. NMR analysis (metabonomics) of urine samples from rats treated with lazabemide (1500 mg/kg) indicated a decreased excretion of succinate, citrate and oxo-glutarate consistent with disruption of mitochondrial respiration (see presentation by U. Niederhauser et al.). As lazabemide is also a substrate for mitochondrial monoamine oxidase it was hypothesized that lazabemide induced hepatotoxicity is related to oxidative stress conditions likely due to a production of reactive products capable of mitochondrial function impairment. Treatment of rat hepatocytes in primary culture with 300 µM lazabemide did not lead to measurable cytotoxic effects. However, pre-treatment of cells with buthionine-sulfoximine and diethyl-maleate depleting reduced cellular glutathione and inhibiting its synthesis markedly increased cytotoxicity as assessed by the release of lactate dehydrogenase into the incubation medium. Thus, reduced glutathione seems to play an essential protective role against lazabemide-mediated cytotoxicity. Additional experiments aimed at detecting reactive species originating from lazabemide and its metabolism will be presented to clarify the role of oxidative stress response on mitochondrial function.

961 METABONOMICS STUDY INTO THE BIOCHEMICAL EFFECTS OF RO 19-6327 USING ^{1}H NMR SPECTROSCOPY AND PATTERN RECOGNITION ANALYSIS OF URINE SAMPLES.

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Tempium® (Lazabemide: Ro 19-6327) is a potent, highly-selective and reversible inhibitor of MAO-B with possible clinical advantages over current therapies for the treatment of Alzheimer's Disease. Results of toxicity studies showed that Ro 19-6327 induces in rats after repetitive oral doses of ≧ 700 mg/kg a mild transient increase in liver-enzymes (mostly ALT) and an increase in liver weight. A similar trend for an increase in liver-enzymes was observed in patients. To evaluate the technique and to gain further insight into the effects of Ro 19-6327, ^{1}NMR spectroscopy and metabonomics investigations have been performed using urine samples of Wistar rats. Two dose levels (single oral doses of 600 mg/kg and 2000 mg/kg) were given to assess the sensitivity of the metabonomics approach, and to evaluate if there are quantitative as well as qualitative changes in the toxicity profile. Ro19-6327 caused subtle biochemical perturbations in the urinary ^{1}H NMR spectra. The major changes in the urinary profiles were, beside the appearance of drug related resonances, severe reduction of citrate, 2-oxoglutarate and succinate. Depletion of these TCA cycle intermediates may be indicative of mitochondrial dysfunction. The subtle elevation of creatinine and taurine excretion may be associated with effects of Ro19-6327 on the liver. Little difference in the biochemical response of rats treated with 600 or 2000 mg/kg of Ro 19-6327 was observed. However, rats treated at the higher dose showed a more prolonged response. The effects were quickly reversible, with urinary profiles returning to normal by 3-4 days post-dose. Conclusion: Metabonomics may add valuable information to assess drug-induced effects. The results also confirm that Ro19-6327 exhibits low toxicity in the rat.

962 IMPROVED CLASSIFICATION OF HEPATIC TOXICITY BASED ON PATTERN RECOGNITION INVESTIGATION OF DIFFUSION-EDITED 1H NMR SPECTRA OF BLOOD SERUM.

B. M. Beckwith-Hall, E. Holmes, N. Thompson, J. C. Lindon and J. K. Nicholson. *Biological Chemistry, ICMS London University, South Kensington, London, United Kingdom.* Sponsor: D. Robertson.

Chemometric investigation of 1H NMR biofluid spectra taken from animals dosed with model toxins generates characteristic patterns of metabolic responses and enables the identification of biomarkers of toxic response and regeneration. Most of the studies to date have been conducted on urine, with limited investigations using blood plasma. In the current study we demonstrate new experimental methods for investigating toxin-induced changes in plasma profiles using ANIT (α-naphthylisothiocyanate, a model cholestatic agent). The use of conventional 1-dimensional 1H NMR spectra generates complex spectra with each biofluid requiring tailored NMR experiments and processing to optimize biological interpretation. Biofluids such as blood plasma or serum contain a wide range of macromolecules that generate a broad envelope of NMR signals, on which are superimposed sharper signals from lower mw metabolites and thus, key biomarker metabolite resonances can be obscured. ANIT was administered to male SD rats (@ 200 mg/kg p.o.) and plasma samples obtained prior to and at 48 h and 72 h post dosing. In order to simplify the subtle ANIT-induced changes in the lipoprotein profile of serum, diffusion-edited spectroscopy coupled with principal components analysis was used to focus on toxin-induced lipoprotein changes by minimizing the contribution of low mw molecules to the spectral profile. Using difusion edited spectra, marked changes to the plasma lipid profiles were detected and good separation of control and treated animals was effected. This study has demonstrated the potential of the application of chemometric analysis to diffusion edited 1H NMR spectra for probing lipoprotein metabolism disorders associated with toxic lesions.

963 NMR-BASED METABONOMIC STUDIES ON SALT TOXICITY IN ZEA MAYS: DECONVOLUTION OF PHYSIOLOGICAL AND TOXICOLOGICAL FACTORS.

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High salinity, whether natural or induced by agricultural methods, is a widespread environmental stressor that can affect development and growth of plants. We have shown that application of NMR-based metabonomic technology can be used to characterise saline-induced stress effects in Zea Mays and to identify pertinent biomarkers. Interpretation of NMR-based metabonomic toxicity studies may be confounded by non-related physiological metabolic variation. Statistical experimental design was used to examine the relationship between metabolic differences arising from growth effects and salt toxicity. Maize plants were subjected to a continuous dosing of either 0, 50 and 150 mM saline solution and harvested after 2, 4 and 6 days (n=5 per class). 1H NMR spectroscopic analysis of the root and shoot extracts indicated differences in the metabolite spectral profiles relating to saline-induced stress. Clustering of control and treated classes based on inherent biochemical differences in the saline-treated plants was achieved *via* application of Principal Components Analysis to the spectral data. Analysis of the control and treated groups revealed clustering according to the time of harvesting, indicating differences in the metabolite profile resulting from growth effects. Partial Least Squares Analysis was employed to deconvolve the biochemical effects of growth and salt stress. Examination of the regression coefficients revealed important metabolic markers for saline toxicity including perturbed levels of malic acid, glucose, alanine, sucrose and glycinebetaine. This NMR-based metabonomic technology provided an efficient method for identifying stress effects in plants. Furthermore, statistically significant biomarkers for salt toxicity, growth differences and the interaction of these physiological factors were identified.

964 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPIC AND PATTERN RECOGNITION STUDIES OF 3-NITROPROPIONIC ACID-INDUCED NEURONAL DAMAGE.

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3-Nitropropionic acid (3NP) is a neurotoxin that produces lesions within the basal ganglia (particularly targetting the striatum) in rats and non-human primates with symptoms mimicking Huntington's Disease. 3NP is also an irreversible inhibitor of succinate dehydrogenase. Nuclear magnetic resonance (NMR) spectroscopy combined with pattern recognition (PR) analysis of biofluids and tissues offers a powerful approach to the investigation of the molecular consequences of toxin exposure. In this study magic angle spinning (MAS) NMR-PR methods have been applied to characterise the metabolic alterations in various neuronal regions caused by 14 days repeated exposure to 3NP (0, 5.0 and 7.5mg/kg i.p.) in the Sprague-Dawley rat. Selected brain regions were dissected and immediately snap frozen before analysis by 1D MAS-NMR. Principal components analysis (PCA) was used to show the biochemical variations induced by 3NP toxicity as a time-related trajectory plot. From the PCA eigenvector loadings, the discriminating regions of the NMR spectra, and thus the contributory endogenous metabolites were identified. Reductions in lactate, acetate, NAA, GABA and creatine levels were observed together with increases in succinate following systemic administration of this toxin. These studies show that MAS-NMR-PR offers a powerful contribution for the investigation of basic mechanisms of neurotoxicity.

965 COMPARISON OF THE TEMPORAL TOXICITY OF HYDRAZINE IN RAT AND MOUSE USING NMR-BASED METABONOMICS.

M. Bollard, T. Ebbels, H. Antti, H. Keun, O. Beckonert, E. Holmes, J. C. Lindon and J. K. Nicholson. *Biological Chemistry, ICMS London University, South Kensington, London, United Kingdom.* Sponsor: D. Robertson.

As part of our ongoing COnsortium for MEtabonomic Toxicology (COMET) designed to systematically investigate metabolic effects of drugs, 1H NMR spectroscopic and pattern recognition (PR)-based methods were used to investigate the biochemical responses to toxins in rat and mouse models. Principal components analysis (PCA) was used to evaluate the metabolic effect of hydrazine at 30 and 90 mg/kg in the rat and at 100 and 250 mg/kg in the mouse. Urine samples were collected from dosed and control animals at 10 time-points over an 8 day period and analysed by 600 MHz 1H NMR spectroscopy. Urinary biomarkers of hydrazine toxicity of the two species were determined from the PC loadings and included a number of endogenous metabolites which were common to both, including 2-aminoadipate, taurine, creatine and the TCA cycle intermediates. Metabolic trajectories were mapped in PC space using the mean spectra at each time point providing a means of monitoring the progression of and recovery from the toxic lesion. Due to the disparate magnitude of response shown in the two species, it was difficult to combine the two data sets in a single chemometric model. Thus, a hierarchical method was developed using multiple partial least squares (PLS) regression models which took account only of the direction of response in metabonomic space rather than magnitude. This enabled the time course of the responses to be compared in a meaningful way, showing that, although there were many similarities in

the effect of hydrazine dosing on endogenous metabolites, there were also subtle differences between the two species. Incorporation of these chemometric methods into automated NMR-based metabonomics will enable toxicological screening of biofluids from different species, thus aiding determination of the model species that best mimic the human.

966 A METABONOMIC INVESTIGATION OF THE BIOCHEMICAL EFFECTS OF ROSIGLITAZONE AND WY14643.

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The effects of diabetic treatment drugs Rosiglitazone and WY14643 were investigated by NMR-based metabonomic analysis using groups of healthy male Sprague-Dawley rats (n=10). 1H NMR spectra of plasma samples were obtained and data filtering and pattern recognition analysis applied to the spectra, in order to establish baseline reference effects of these drugs with particular emphasis on potential toxicological responses in non-disease state animals. Samples obtained after a low dose of Rosiglitazone administration were differentiated from the control group by increases in several metabolites including lipoproteins, triglycerides and glucose, whereas the intermediate group reversed this change and was most similar to the control group. The high dose group presented changes in the opposite metabolic direction to that of the low dose group, with lowered lipids and glucose levels. The WY14643 response also exhibited differential dose-related effects, initially at low dose showing increased lipids and decreased glucose signals. The intermediate-dose group showed an effective reversal of this pattern, and the high dose group was the closest to the control group, though marginally higher in glucose, choline and VLDL levels. This metabonomic evaluation of drug-response is aimed at allowing a better understanding and characterisation of the metabolic responses of diseased animals to these drug treatments, and in particular to identify potential biomarkers of toxic responses in the metabolism of healthy individuals

967 BUILDING NMR SPECTROSCOPIC METABONOMIC DATABASES OF XENOBIOTIC TOXICITY.

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The COnsortium for MEtabonomic Toxicology (COMET) project will construct a database of drug toxicity using ca. 100, 000 ^{1}H NMR spectra of biofluids from animals treated with model compounds. Mathematical models characterising the effects on endogenous metabolites will enable rapid toxicological screening of potential drug candidates and discovery of novel mechanisms and biomarkers for specific types of toxicity. To reveal any systematic differences between the 7 participating animal study centres, hydrazine was administered orally to rats at each site, in two dose groups (30 and 90 mg/kg). Urine samples were collected from dosed and control animals at 10 time-points over an 8 day period. At Imperial College, the urine was analysed by 600 MHz ^{1}H NMR spectroscopy and the spectra used to build statistical pattern recognition (PR) models based on principal component analysis (PCA) and partial least squares (PLS), describing both dose, time and between-Lab. related spectral signatures. The resulting 7 independent datasets showed a high degree of similarity, yet the metabonomic techniques could detect genuine low level biological differences between the laboratories. In addition, ^{1}H NMR spectra of plasma samples collected at 48 and 168 h post dose were analysed using PR. To further refine the information retrieval, PLS was used to model the relation between the NMR spectra and clinical chemistry of the plasma. This revealed co-variation between the NMR and clinical chemistry data, facilitating more detailed interpretation of the metabonomic variation. Finally, the reproducibility of metabonomic methodology was assessed by comparing spectra acquired at two different NMR facilities from alequots of the same urine samples. The two data sets showed close similarity, confirming the robustness of the technique. This work illustrates that metabonomic studies can obtain novel, reproducible information on the toxicity of model, and inevitably new compounds, across multiple laboratories with large numbers of samples.

968 MINIATURIZED NMR PROBEHEAD WITH INCREASED SENSITIVITY FOR PROFILING BIOFLUIDS AND METABOLITE IDENTIFICATION.

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Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful analytical tools to determine the structure of small molecules. It suffers, however, compared to other analytical techniques, from an inherent low sensitivity. Structural characterisation of mass- and volume limited samples are difficult with conventional NMR detectors, e.g.: bio-fluids (CSF, urine, plasma and synovial fluids), drug metabolites, fractionated peaks from micro-separation techniques and isolates of natural products. A new miniaturised 1mm NMR probe was built with a 100-fold smaller active sample volume than conventional NMR probeheads. The new probehead has an active volume of 2.5 µl. With this probe high resolution NMR was performed with hitherto unobtained mass sensitivity. The improvement in the signal to noise ratio by a factor of >4 compared to a conventional system lead to a reduction in measuring time of more than a factor of 16. Furthermore the favourable ratio of analyte to solvent lead to spectra with very low solvent background. NMR results are shown which illustrate the performance of the new probe for metabonomics investigations and metabolite identification. These include 1D 1H NMR spectra of rat urine, plasma and CSF, and 2D NMR experiments of mass limited samples.

969 APPLICATION OF METABONOMICS TO PHARMACEUTICAL DRUG DEVELOPMENT:EVALUATION OF PHENYLACETYLGLYCINE AS A POTENTIAL MARKER OF PHOSPHOLIPID ACCUMULATION.

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Phospholipidosis (PLDS) can be caused by a range of agents in many animal species, including man [1]. PLDS is characterised by the accumulation of phospholipid-rich lamellated and crystalloid inclusions, identifiable only by transmission electron microscopy within a variety of cells [1, 2]. PLDS is a frequent finding in toxicology studies and, in order to miminise impact on drug development, there is a need to develop less labour-intensive and time-consuming methods for its detection. We have used nuclear magnetic resonance (NMR) spectroscopy to help to identify biochemical markers in urine collected from Sprague-Dawley rats following 7 days' repeated dosing with six structurally-related drug candidates (the cationic amphiphiles GSK-A to F) at 3 dose levels. All six compounds in the screen demonstrated morphological changes indicative of phospholipid accumulation in the mesenteric lymph node at the high dose level. Although a number of general housekeeping biochemicals were shown to change in urine by NMR, a feature common to all six drug candidates was a rise in the urinary levels of phenylacetylglycine (PAG). This observation supports previous reports that PAG may constitute a potential marker of PLDS [3, 4]. Significantly, PAG was also present in increased amounts in urine collected from rats dosed for 6 months with a structurally-unrelated drug candidate, GSK-G, that caused PLDS. In this case, PAG returned to control levels in urines collected from these animals after a 3 month off-dose period, mirroring a reversal of PLDS as detected by electron microscopy. This study highlights the utility of metabonomics technology in the monitoring of PLDS as part of novel chemical entity evaluation. Studies are ongoing to elucidate the origin of PAG and its biochemical significance. 1. Toxicologic Pathology 25, 53-60 (1997) 2. CRC Critical Reviews in Toxicology November, 185-217 (1975) 3. Biomarkers 5, 410-423 (2000) 4. Magnetic Resonance in Chemistry 39, 559-565 (2001)

970 APPLICATION OF METABONOMICS IN DRUG DESIGN: CAN METABONOMICS AND PHARMACOKINETICS (PK) STUDIES BE CONDUCTED SIMULATANEOUSLY IN THE SAME SET OF RATS?

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High-resolution ^{1}H-NMR spectroscopic analysis of biofluids is a powerful technique for investigating the response of organisms to xenobiotics. The ^{1}H-NMR spectral profiles of biofluids provide a unique temporal fingerprint of the metabolic state of an organism and information on the nature of xenobiotics to which an organism has been exposed. Metabonomics methods combine high resolution NMR of biofluids with pattern recognition techniques to determine the onset, duration, and localization of a toxic lesion in target organs. The feasibility of simultaneous metabonomics and PK studies in the same rats was assessed. Male Sprague-Dawley rats were divided into two experimental groups, without surgery (normal) or cannulated in the carotid artery (cannulated). Within each group, rats (n=6) received vehicle (control), methotrexate, or acetaminophen. Blank urine was collected on day 0. On day 1, treatments were delivered and the cannulated group was subjected to PK bleeding procedures. Urine was collected twice a day for 7 days. Urinary NMR spectra were acquired and data were analyzed with principal component analysis (PCA) and soft independent modeling of class analogy (SIMCA). Good clustering and good separation of clusters were observed in PCA map for all groups. When treated with xenobiotics, cannulated rats do not cluster the same as normal rats, indicating surgery had an impact on the response to xenobiotics treatment.

Loadings derived from SIMCA models indicate that there is no linear superposition of the effect of cannulation and the effect of xenobiotics exposure. These results suggest that simultaneous PK and metabonomics studies are feasible, which will provide information on both the systemic exposure and the effect of xenobiotics on the metabolic state of the rats. However, this requires a separate database for toxicity prediction built using cannulated rats.

971 COMPARISON OF NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY OF URINE AND CONVENTIONAL PATHOLOGY MEASUREMENTS IN PRECLINICAL SAFETY EVALUATION OF ALLYL ALCOHOL.

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Detection of organ specific toxicity using nuclear magnetic resonance (NMR) spectroscopy of urine may be a novel method for evaluating toxicity of xenobiotics. This technique may allow for detection of toxicity below the doses that induces changes in standard pathology endpoints (e.g., serum chemistry and histopathology). The present study assessed this proposition using the model hepatotoxicant, allyl alcohol. Four Sprague-Dawley rats of each sex were orally gavaged with allyl alcohol at 0, 60, 120, or 240 mg/kg on Day 1 of the study. Survival permitting, two animals of each sex were necropsied on Days 3 and Day 5. Twenty-four hour urine samples were collected in metabolic cages prior to dosing and daily through Day 5. NMR spectra were acquired and the data subjected to principal component analysis (PCA). At necropsy, blood was taken for serum chemistry analysis, and selected organs were processed for microscopic evaluation. Allyl alcohol induced significant, dose-dependant increases in total bilirubin, blood urea nitrogen, release of hepatocellular enzymes (ALT, AST, LDH), and globulin as well as reducing serum albumin. The three most common histologic lesions were hepatocellular necrosis, forestomach ulceration, and granulocytic hyperplasia. NMR analysis of urine reliably separated samples from treated animals from those of control and pre-dose samples. The PCA of hematology and clinical chemistry showed similar pattern without time-dependent information. Allyl alcohol (60 mg/kg) increased urinary taurine levels on Day 1, with partial recovery by Day 5. The extent of NMR changes was correlated with the severity of hepatocellular necrosis. The NMR PCA revealed that inter-animal differences exceeded intra-animal differences. These data suggest two main roles for NMR spectroscopy in preclinical safety assessment: noninvasive metabonomic analysis of novel xenobiotic toxicity, and selection of metabolically homogenous animal population for *in vivo* studies.

972 EVALUATION OF METABONOMIC TECHNOLOGY FOR LIVER AND KIDNEY TOXICANTS IN MICE.

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Metabonomic technology has been well characterized for the rat, however limited information is available for mice. The purpose of this study was to evaluate the utility of the mouse as a test system for early toxicology screening using metabonomics. Cisplatin (CP), 2-bromoethylamine (BEA), carbon tetrachloride (CCl_4), and p-aminophenol (PAP) were used as representative liver and kidney toxicants. Male B6C3F1 mice (4/dose group) were individually housed in metabolism cages and urine collected pre-test and every 24 hr period through Day 8. A low and high dose for each toxicant was selected to assess a Dose-Response effect. CP (10 and 25 mg/kg), BEA (100 and 30 mg/kg), CCl_4 (3000 and 2400 mg/kg) and PAP (100 and 300 mg/kg) were administered as single intraperitoneal or oral doses. Clinical chemistry and selected target organ histopathology was collected Day 2 and Day 8. High-resolution 1H-NMR spectra from urine were collected and data subjected to principle component analysis (PCA). A clear dose and time response was observed in the PCA analysis of the urine spectra with changes in agreement with clinical chemistry parameters in high-dose groups. In low-dose treated CP animals, PCA demonstrated effects at low doses which were not evident by clinical chemistry. PCA also demonstrated a time course of response, with early toxicity occurring followed by apparent recovery. These data demonstrate that the mouse is a viable test system for predictive toxicology screening using metabonomic technology.

973 APPLICATION OF NMR-BASED METABONOMIC TECHNOLOGY TO TOXICITY SCREENING; CHARACTERIZATION AND PREDICTION OF NEPHROTOXICITY.

J. T. Brindle, E. Holmes, J. K. Nicholson and J. Connelly. *Biological Chemistry, Imperial College, S. Kensington, United Kingdom.* Sponsor: D. Robertson.

NMR-based metabonomic technology was applied to the characterization of tissue and mechanism specific renal toxicity. Acute nephrotoxicity was induced in male SD rats using a single dose of one of the following; vancomycin, ifosfamide, lead acetate, maleic acid, sodium chromate and sodium fluoride. Proton NMR spectroscopic analysis was performed on urine samples obtained at timed intervals following dosing. Data reduction methods and multivariate statistical analyses were applied to the spectral data and separate mathematical models constructed to characterize the metabolic effects and biomarkers of each toxin. These models were combined to form a simple expert system for classifying nephrotoxicity. Extension of this preliminary expert system will be of obvious benefit for lead compound selection in the pharmaceutical industry.

974 MULTIVARIATE STATISTICAL PRE-TREATMENT OF NMR-BASED METABONOMIC DATA TO REMOVE INHERRANT PHYSIOLOGICAL VARIATION CONFOUNDING TOXICITY PATTERNS.

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Proton NMR-based metabonomics is a well-established technique used to analyze and interpret complex multiparametric metabolic data and has a wide number of applications in the development of pharmaceuticals. However, interpretation of biological data can be obscured by extraneous variation such as physiological variation or confounding toxicity related changes. Here we have shown the novel application of a data filtering method, orthogonal signal correction (OSC), to biofluid NMR data to minimize innate physiological variation and confounding toxicity patterns in two independent studies. The removal of this orthogonal variation exposed features of interest in the NMR data and facilitated biological interpretation and identification of biomarkers of toxicity. Furthermore, analysis of the orthogonal variation provided an explanation of the systematic spectral changes responsible for confounding the relevant toxicity patterns.

975 RENAL ORGANIC ANION TRANSORTER IS A MECHANISM FOR THE RENAL UPTAKE OF MERCURIC CONJUGATES OF CYSTEINLY HOMOLOGS.

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The role of the human organic anion transporter subtype 1 (hOAT1) in the uptake of mercuric conjugates of cysteine (CYS), N-acetylcysteine (NAC) or GSH was studied in an MDCK cell-line stably transfected with the open reading frame sequence of the hOAT1 transporter. Uptake of p-aminohippurate (PAH), which is a high-affinity substrate for OAT1, by the transfected cells was 20 times greater than that in the non-transected cells. Mercuric conjugates, especially of CYS and NAC were significantly more toxic in hOAT1-transfected cells than in non-transfected cells as determine by a decrease in mitochondrial dehydrogenase activity (MTT conversion). This was particularly the case for the mercuric conjugate of NAC, which induced greater than 50% cellular death over 18 h at a concentration of 100 microM. The toxic effects of the mercuric conjugate of NAC was prevented by co-exposure to probenecid (a competitive inhibitor of OAT1) and partially prevented by co-exposure to PAH. Toxicity was also cis-inhibited by the OAT1 exchangeable dicarboxylates alpha-ketoglutarate, glutarate, and adipate. However, succinate, a non-exchangeable dicarboxylate, did not cis-inhibit induction of toxic effects. Compared with the toxicity induced by unbound mercuric ions, which occurred steadily over a 24-hour time period, no apparent toxic effects were detected with mercuric conjugates of NAC until ten hours of exposure had elapsed. Uptake studies confirmed a direct relationship between uptake of mercury and the toxic effects elicited. Probenecid, PAH and dicarboxylates competitively inhibited the uptake of mercuric conjugates of CYS or NAC. These findings are the first real line of mechanistic evidence showing that OAT1 transports mercuric conjugates CYS and NAC, which are species of mercury taken up *in vivo* and vitro by proximal tubular epithelial cells, which are the main targets of inorganic mercury in the kidneys.

976 INORGANIC MERCURY TOXICITY AND TRANSPORT IN DISTAL SEGMENTS OF THE RABBIT NEPHRON.

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Luminal transport and acute toxicity of inorganic mercury in selected segments of the distal region of the rabbit nephron were studied using the isolated perfused tubule technique. Segments studied were the cortical thick ascending limb (cTAL)

and the cortical collection duct (cCD). Both segments were relatively resistant to the toxic effects of luminal inorganic mercury (^{203}Hg, 9.3μM for the cCD and 20μM for cTAL) when compared to its acute toxic effects on the proximal tubule, which are prominent cellular uptake of vital dye, cellular swelling, marked luminal membrane blebbing and a great increase in the leakiness of the tight junctions. The cCD showed no acute toxic effects from luminal mercury while the cTAL was sensitive to the presences of the luminal inorganic mercury with some slight blebbing of the luminal membrane and an increase in the leakiness of the tight junctions, but there was no cellular swelling nor cellular uptake of the vital dye. The transport of luminal inorganic mercury was measured by its disappearance rate from the lumen (JD, fmol min^{-1} (mm tubular length)$^{-1}$), its appearance rate in the bath (JA, fmol min^{-1} (mm tubular length)$^{-1}$) and by cellular accumulation (fmol (mm tubular length)$^{-1}$). The JD was 33.5 ±2.8 and 81.6 ±9.5 for the cCD and cTAL respectively, while the JA was 9.3 ±2.1 and 70.0 ±17.4 for the cCD and cTAL respectively. The asymmetry of the JD and JA within each segment may be due to cellular uptake of the mercury, which was 1, 342± 347 and 1, 526 ±164 in the cCD and cTAL respectively. For both the cCD and cTAL, approximately 80% of the cellular accumulated mercury was associated with the tubular structure and 20% with the cytoplasm. We conclude that the cCD and the cTAL can transport inorganic mercury from the luminal fluid. A significant fraction of the accumulated mercury in the cell may be due to non-specific binding. Also, these segments are relatively resistant to the acute toxic effects of inorganic mercury.

977 MERCURIC ION (HG^{2+}) INCREASES THE SENSITIVITY OF KIDNEY CELLS TO APOPTOSIS BY INHIBITING NUCLEAR FACTOR-κB (NF-κB) ACTIVATION.

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NF-κB is a thiol-dependent transcriptional factor that promotes cell survival and protects from apoptotic stimuli. Recently, we reported (TAP 173:176-187, 2001) that Hg^{2+}, one of the strongest thiol-binding agents known, impairs NF-κB activation and transcriptional activity in normal rat kidney epithelial (NRK52E) cells at low concentrations (< 20 μM) by binding to specific reduced thiol moieties in the NF-κB activation pathway. Since NF-κB prevents apoptosis, we hypothesized that attenuation of NF-κB activation by Hg^{2+} may increase the sensitivity of kidney cells to apoptotic agents to which kidney cells are otherwise resistant because of their NF-κB activating capacity. In untreated cells, fewer than 5% were apoptotic when evaluated by DNA fragmentation (TUNEL) or flow cytometric DNA profile analyses. Hg^{2+} (5 μM) treatment for 24 hrs increased this proportion by 1.5- to 2-fold. Neither lipopolysaccharide (LPS) (1 μg/ml) nor tumor necrosis factor-α(TNF) (100 U/ml), potent inducers of NF-κB, altered the proportion of apoptotic cells, compared with untreated controls. However, when LPS or TNF were given following Hg^{2+} treatment (5 μM for 30 min.), the proportion of cells undergoing apoptosis at 22 hrs increased by 4- to 6-fold over that seen following LPS or TNF alone. In contrast, Hg^{2+} pretreatment did not increase the amount of apoptosis caused by apoptosis-inducers that do not activate NF-κB (staurosporine, Fas ligand). These findings support the view that Hg^{2+} enhances the sensitivity of kidney cells to apoptotic stimuli as a consequence of inhibition of NF-κB activity. Since apoptosis is known to play a key role in the pathogenesis of renal failure resulting from toxicant injury to proximal tubular cells, promotion of apoptosis *via* inhibition of NF-κB activity may define a novel molecular mechanism by which Hg^{2+} toxicity is initiated in kidney cells. Supported by ES04696 and ES07033 from the NIH.

978 DIFFERENTIAL SENSITIVITY TO CHEMICALLY INDUCED INJURY IN PRIMARY CULTURES OF RENAL EPITHELIAL CELLS FROM CONTROL AND UNINEPHRECTOMIZED RATS.

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Primary cultures of renal proximal tubular (PT) and distal tubular (DT) cells from rats that had undergone uninephrectomy and compensatory renal growth (NPX) retain changes in morphology, enzyme and transport activities, and mitochondrial function that occur in kidneys of NPX rats. Acute cytotoxicity of tert-butyl hydroperoxide (tBH; 0.1, 0.5, 1, 5, 10 mM) was determined by measurement of lactate dehydrogenase (LDH) release in both freshly isolated and primary cultures of renal PT and DT cells from control and NPX rats. tBH-induced death of both cell types from NPX rats was greater than that in corresponding cells from control rats (net LDH release = 33.6% *vs.* 53.7% for PT cells from control and NPX; 10.8% *vs.* 62.1% for DT cells from control and NPX). tBH-induced malondialdehyde formation was 2- to 4-fold greater in PT and DT cells from NPX rats than in cells from control rats. Similarly, tBH and KCN (0.1-1 mM; a mitochondrial toxicant) both induced significantly greater cell death in confluent primary cultures of PT and DT cells from NPX rats than in corresponding cells from control rats. For inorganic Hg (5, 10, 50 μM), however, cytotoxic effects in cells from NPX rats were similar to those in corresponding cells from control rats at the two lower concentrations but were markedly higher at the highest concentration. Uptake of inorganic Hg (0.1, 1, 5 μM) across the brush-border and basolateral membranes of PT and DT cells grown on filter inserts was actually lower in cells from NPX rats at the two lower doses but was higher at the highest dose as compared to measurements in cells from control rats. These results indicate a general greater sensitivity of cells from NPX rats to chemical toxicants than cells from control rats. For inorganic Hg, however, more complex dose-response relationships exist between transport and toxicity. (Supported by NIEHS Grant ES05157, ES05980, and DK40725.)

979 IS MERCURY IN URINE INDICATIVE OF EXPOSURE TO LOW LEVELS OF MERCURY VAPOR?

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Exposure to elemental mercury has received increased attention as a result of ubiquitous use, improved analytical detection, and awareness of health effects. Although urinary mercury levels are considered the best predictor of mercury vapor exposures, a lack of relationship has been reported between airborne and urinary mercury levels at low air levels (i.e., <50 $μg/m^3$). We reviewed the literature on the correlation of mercury in air and urine to evaluated whether airborne mercury exposures at health-based action levels (e.g., 1 $μg/m^3$) are measurable in urine above background. A meta-analysis was conducted of ten studies reporting paired air and urine mercury data (149 samples total) and meeting specified criteria for data quality and sufficiency. The log-transformed dataset showed a strong correlation between mercury in air and in urine (r=0.77), although the relationship was best fit by a series of parallel lines with different intercepts for each study (R^2=0.81). Predicted ratios of air to urine mercury levels at 50 $μg/m^3$ air concentration ranged from 1:1 to 1:3. Toward the lower end of the dataset (i.e., 10 $μg/m^3$), predicted urinary mercury levels encompassed two distinct ranges: values on the order of 20 μg/L and 30–60 μg/L. Extrapolation to 1 $μg/m^3$ resulted in predicted urinary levels of 4–5 and 6–13 μg/L. Higher predicted urine mercury levels were associated with use of static area air samplers by some studies, rather than more accurate personal air samplers. Urinary mercury predictions based primarily on personal air samplers at 1 and 10 $μg/m^3$ are consistent with reported mean (4 μg/L) and upper-bound (20 μg/L) background levels, respectively. Thus, although mercury levels in air and urine are correlated below 50 $μg/m^3$, the impact of mercury in air below 10 $μg/m^3$ is likely indistinguishable from background urinary mercury levels.

980 MERCURIC CHLORIDE SUPPRESSES CYCLOOXYGENASE-2 EXPRESSION AND PROSTAGLANDIN PRODUCTION IN A HUMAN MONOCYTIC CELL LINE.

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We examined the effects of a brief treatment of low-dose mercuric chloride on the activation of arachidonic acid metabolism in the human monocytic cell line THP-1. HPLC analyses revealed that LPS treatment stimulated induction of cyclooxygenase-2 expression and production of prostaglandin E2 (a major arachidonate metabolite)in these cells. Unstimulated THP-1 cells express the constitutive cyclooxygenase-1 isoform and a low level of cyclooxygenase-2, with a basal production of prostaglandin E2. THP-1 cells were pre-treated with 1 microM mercuric chloride for one hour, and then were incubated with LPS (1 microg/ml) for 4 hours. Cell viability was not affected by treatment with inorganic mercury, as assessed by trypan blue exclusion. Treatment with mercuric chloride suppressed both the basal level and LPS-stimulated expression of cyclooxygenase-2, as measured by Western-blot analysis. Correspondingly, EIA analysis revealed that both basal and LPS-stimulated prostaglandin E2 synthesis was attenuated by mercuric chloride treatment. We did not observe alteration of cyclooxygenase-1 expression. These results demonstrate an effect of mercury on attenuating the arachidonic acid pathway in activated monocytes/ macrophages. Modulation of this pathway by inorganic mercury suggests that part of the adverse effects of exposure to low levels of this metal may be suppression of inflammatory and immune responses.

981 EFFECTS OF METHYLMERCURY EXPOSURE ON THE IMMUNE AND NERVOUS SYSTEM RESPONSES OF CBA/J MICE TO A CHRONIC TOXOPLASMA GONDII INFECTION.

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Previous studies from our laboratory suggest that exposure to methyl mercury (MeHg) does not increase the susceptibility to acute toxoplasmosis in CBA/J mice. Therefore, we investigated endpoints associated with immunotoxicity and neuro-

toxicity in 6-week old, female CBA/J mice exposed to both MeHg and T. gondii during a chronic T. gondii infection. Mice were fed 25 T. gondii tissue cysts and dosed six weeks later with 20 mg/kg of MeHg. There were significant differences ($P < 0.05$) within the T cell subpopulation ratios as indicated by flow cytometric analysis. MeHg increased splenic CD8+ T cell subpopulations with or without a concurrent T. gondii infection, while the CD4+ T cell subpopulations were decreased ($P<0.05$). MeHg had no effect on the CD4+CD8+ T cell or non-T cell subpopulations. Groups without T. gondii infection showed a decrease in CD8+ T cells when compared to groups with the infection, while there was no difference in CD4+ T cells between infected or non-infected mice. There was a significant ($P<0.05$) increase in brain tissue cyst counts within the group exposed to both MeHg and T. gondii (16 ± 4, mean ± SE, n=7) *versus* T. gondii alone (4 ± 1, n=8). These data indicate that concurrent exposure to both MeHg and T. gondii has notable effects, especially on the immune system (Supported by NIH Grant F36GM20301).

982 DECREASED ARGINASE ACTIVITY AND LOSS OF MANGANESE CONTENT OF RAT LIVER BY METHYLMERCURY EXPOSURE *IN VIVO*.

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Sponsor: A. Naganuma.

Alteration in arginase (ARI) which is an abundant manganese-binding protein in liver during methylmercuric chloride (MMC) exposure in vivo was examined because our previous study indicated that MMC administration caused a decrease in the mangaese-superoxide dismutase activity without affecting its gene expression and protein content. Subcutaneous administration of MMC (10 mg/kg/day) for 5 days to rats resulted in drastic decrease in the ARI activity (44% of control). Under the condition, hepatic manganese content did decline to 49% of control. Interestingly, there was a strong positive correlation ($r=0.72$, $P<0.01$) between hepatic ARI activities and hepatic manganese levels. Immunoblot analysis revealed that reduction of ARI activity caused by MMC was not due to that of the protein content. With purified ARI it was found that an thiol-modifying agent N-ethylmaleimide as well as MMC suppressed the enzyme activity, but this inhibitory effect of these chemicals reached plateau levels (20-30% of control), suggesting contribution of sulfhydryl modification to the decrease in ARI activity. This hypothesis was further supported by steady-attachment of ARI to an agarose gel conjugated with arylmercury and then dissociation of this protein from the affinity resin by 2-mercaptoethanol, but not NaCl, nonionic detergent and basic amino acid. These results suggest that covalent interaction of MMC with ARI through accessible cystein residues, presumably resulting in manganese release from the active center, appears to be, at least partially, associated with MMC-mediated reduction of ARI activity observed *in vivo*.

983 INORGANIC MERCURY SUPPRESSES NITRIC OXIDE PRODUCTION AND MODULATES CYTOKINE GENE EXPRESSION THROUGH NF-κB SIGNALING PATHWAY IN MURINE MACROPHAGE.

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Mercury is a heavy metal known to adversely affect the immune system; however, little is known regarding molecular mechanisms. Previous studies showed that mercury potently inhibits nitric oxide (NO) production in pancreatic islet β-cells. Macrophages are major producers of NO and this signaling molecule is important in the generation and regulation of immune responses. Therefore, macrophages may be a target for mercury intoxication. The mechanisms involved in the regulation of NO by mercury remain unclear. We investigated the hypothesis that mercury inhibits NO production through a nuclear factor κB (NF-κB)-dependent mechanism. In this study J774A.1 cells, a murine macrophage cell line, were used to investigate the effects of low dose exposure to inorganic mercury (Hg) on the production of NO, TNFα, IL-6 and IL-1β. Cells were stimulated with Hg with or without LPS (30 ng/ml). Hg (5-20 μM) dose-dependently decreased the production of NO in LPS-stimulated cells. Concomitant decreases in the expression of inducible nitric oxide synthase (iNOS) mRNA and protein were observed. Treatment of J774A.1 cells with Hg alone did not affect the production of NO nor the expression of iNOS mRNA or protein. Interestingly, Hg stimulated the expression of TNFα mRNA and increased LPS-induced TNFα and IL-6 mRNA expression measured by reverse transcription-polymerase chain reaction. IL-1β mRNA expression was not affected by inorganic mercury. NF-κB controls the expression of many immune related genes including iNOS and pro-inflammatory cytokines. Exposure to Hg (20 μM) inhibited LPS-induced nuclear translocation of NF-κB as determined by western blot using anti-p65 antibody. Inhibition of NF-κB activity represents a likely mechanism for the inhibitory effects of Hg on NO production but fails to explain the stimulatory effects of this metal on cytokine gene expression. Other signaling pathways are currently under investigation, namely the effects of Hg on p38-mitogen activated protein kinase.

984 INORGANIC MERCURY (HGCL2) DECREASES ADIPOGENESIS AND CAUSES INSULIN RESISTANCE IN 3T3-L1 ADIPOCYTES.

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Many aspects of the differentiation process of the 3T3-L1 preadipocyte fibroblast cell line have been determined and can be used as a model to study the effects of toxins on adipogenesis. Mercury (Hg) is a prevalent and mobile toxin in our environment that has been shown to increase the glucose uptake of mature adipocytes. In this report, we examine the effects of Hg on adipocyte differentiation and insulin responsiveness. 3T3-L1 fibroblasts were differentiated in the presence or absence of 1-10 μM Hg. The presence of Hg decreased the number of cells differentiating to adipocytes, decreased their lipid content, and decreased expression of PPARγ. Lipid content of cells differentiated in the presence of 5 or 10 μM Hg decreased by 22 and 21% respectively. Hg reduced the number of differentiated adipocytes by 14, 36 and 55% for 5, 10 and 20 μM Hg respectively. PPARγ expression decreased 60% in the presence of 5 and 10 μM Hg. Another physiological consequence of Hg exposure during differentiation was a reduction of insulin mediated glucose uptake. Cells differentiation in the presence of 1, 5, and 10 μM Hg decreased their insulin-mediated glucose uptake by 25, 57, and 62% respectively. These results show that low levels of Hg can disrupt adipocyte differentiation and alter their subsequent physiological responses to a hormonal stimulus.

985 MERCURY CONTAMINATION IN INTERNAL ORGANS OF CETACEANS MARKETED FOR HUMAN CONSUMPTION.

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The internal organs such as liver, kidney, lung, and small intestine of toothed whales and dolphins have been traditionally eaten in the limited area of Japan. A mixture of these organs is sold as boiled products. We surveyed Hg contamination in the internal organs of cetaceans sold in Japanese market for human consumption. The averages with SD of total mercury (T-Hg) were 357 ± 520 (0.195-1980, n=27) ppm in liver, 40.5 ± 48.5 (7.85-95.1, n=15) ppm in kidney and 42.8 ± 43.8 (2.10-79.6, n=23) ppm in lung. The provisional limit of T-Hg in marine products set by Japanese Ministry of Health and Welfare is 0.4 ppm. The highest of T-Hg detected in the liver was about 5000 times higher than their limit, suggesting acute intoxication of Hg even by a single consumption. A high correlation was observed between T-Hg and Se concentrations; the molar ratio of T-Hg/Se in these organs was slightly higher than 1.0. Furthermore, a significant correlation was observed between Zn and Cu concentrations in liver and lung, suggesting the induction of metallothionein (MT). Probably, the formation of Hg-Se complex and the induction of MT should decrease the toxicity of Hg for cetaceans and allow tremendous accumulation of Hg in their livers. Supported by IFAW.

986 GADOLINIUM PRETREATMENT PREVENTS CADMIUM-INDUCED LIVER DAMAGE IN BOTH WILD-TYPE AND METALLOTHIONEIN-NULL MICE.

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The heavy metal cadmium (Cd) causes hepatotoxicity upon acute administration. Kupffer cells, the resident macrophages of the liver, have been implicated in the mechanism of Cd-induced hepatotoxicity, as suppressing Kupffer cells with gadolinium chloride ($GdCl_3$) prevents Cd-induced hepatotoxicity. However, $GdCl_3$ may also induce the Cd-binding protein, metallothionein (MT). Therefore, a study was conducted to determine whether $GdCl_3$ prevents Cd-induced hepatotoxicity *via* the induction of MT or depletion of Kupffer cells. First, hepatic MT and Kupffer cell counts were analyzed 24 h after administration of saline or 10, 30, or 60 mg $GdCl_3$/kg to wild-type (WT) mice. Second, WT and MT-null mice were pretreated with saline or 10, 30, or 60 mg $GdCl_3$/kg 24 h prior to a hepatotoxic dose of Cd (2.5 mg Cd/kg) to examine whether the hepatoprotective effects of

$GdCl_3$ are related to the observed induction of MT or Kupffer cell activation. Blood and livers were removed 16 h after Cd administration and analyzed for hepatotoxicity as well as MT and Kupffer cell content. $GdCl_3$ treatment alone induced MT in a dose-dependent manner and did not affect hepatic non-protein sulfhydryl content. Cd-induced hepatotoxicity was prevented in both WT and MT-null mice that were pretreated with 30 or 60 mg $GdCl_3$/kg, demonstrating that MT induction is not required for the hepatoprotective effects of $GdCl_3$. Hepatic Cd content was not decreased by $GdCl_3$, demonstrating that $GdCl_3$ did not alter Cd distribution to the liver. Furthermore, Kupffer cells were depleted at all doses of $GdCl_3$, whereas hepatoprotection was only observed at doses of 30 and 60 mg, but not 10 $GdCl_3$/kg. This did not conclusively rule out Kupffer cells in the mechanism of Cd-induced hepatotoxicity, but it does suggest that $GdCl_3$ exerts hepatoprotective effects on the liver in addition to depleting Kupffer cells. In summary, these data rule out MT induction and suggest a limited role for Kupffer cells as mechanisms of $GdCl_3$-induced protection from Cd-induced hepatotoxicity. (Supported by NIH grants ES-01142 and ES-07079)

987 MECHANISM OF RESISTANCE TO CADMIUM TOXICITY IN MALE WISTAR-IMAMICHI RATS.

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It has been shown that the toxic effect of cadmium (Cd) can be varied with species, strain and sex in experimental animals. For these reasons, one of the key elements is thought to be metallothionein (MT) that plays a critical role in detoxification of Cd. However, the detailed mechanism for these variations of Cd toxicity remains to be elucidated. In the present study, we compared Cd-induced mortality in four strains of male rats. In the case of Fischer, Wistar and Sprague-Dawley strains, a single injection of 5 mg Cd/kg proved very toxic and induced 80~100% mortality within 2 days. On the other hand, in Wistar-Imamichi strain, the Cd treatment proved nonlethal during 7 days. Next, we attempted to analyze the inheritance pattern of Cd resistance, using resistant (Wistar-Imamichi) and sensitive (Fischer) inbred rats to the metal. The resistance to Cd toxicity was also observed in male rats of first filial (F1) generation (male Fischer x female Wistar-Imamichi). In order to establish whether the resistance to Cd toxicity in male Wistar-Imamichi rats is androgen-dependent, the effect of testectomy on Cd-induced mortality was examined. However, testectomy had no effect on the resistance to Cd toxicity in male Wistar-Imamichi rats, suggesting the resistance is independent of the regulation by androgens. Furthermore, we determined GSH and MT levels in several tissues of resistant (Wistar-Imamichi) and sensitive (Fischer) rats. Pronounced difference of GSH and MT levels in these two strains was not observed in the control or Cd-treated rats. Therefore, it is possible to assume that endogenous substance(s) other than GSH and MT is involved in the resistance to Cd toxicity found in male Wistar-Imamichi rats. Further studies are in progress to elucidate the mechanism of the resistance to Cd toxicity in male Wistar-Imamichi rats.

988 INDUCTION OF METALLOTHIONEIN BY CADMIUM IS ASSOCIATED WITH P53 AND APOPTOSIS IN HUMAN EPITHELIAL BREAST CANCER CELLS.

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Although the function of metallothionein (MT), a metal-binding protein, remains unclear, previous studies in human tumors and cellular differentiation have suggested a pro-apoptotic function. Since the tumor suppressor protein p53 plays an important role in apoptosis, the present study was undertaken to investigate any potential relationship between MT and p53 using human breast cancer epithelial cell lines differing in p53 and estrogen-receptor (ER) status. Cells were treated with 10 μM $CdCl_2$ for 24 h, and MT protein and mRNA levels were measured by ^{109}Cd-heme assay and RT-PCR. MCF7 cells (p53+, ER+) showed a much greater induction in MT protein and mRNA expression as compared to cell lines which are p53- and ER- or weakly positive for ER (ER±). MT localization was determined by immunohistochemical staining. Prior to Cd treatment, MCF7 and HCC1806 (p53-, ER-) cells both showed mainly cytoplasmic staining. Following treatment, MCF7 cells showed intense nuclear and cytoplasmic staining while HCC1806 cells showed staining in the cytoplasm and weak staining in the nucleus. The TUNEL technique was used to study Cd-induced apoptosis. It was found that 10-40 μM $CdCl_2$ treatments caused significant apoptosis in MCF7 cells, particularly at 6-8 and 48 h, but not in MDA-MB-231 cells (p53-, ER-). The data demonstrate that the induction of MT expression is higher in p53+ cells such as MCF7 than p53- epithelial cells. It also suggests that Cd-induced apoptosis may occur mainly in p53+ cells and could be a p53-dependent pathway. Since these cells can induce MT activity after exposure to Cd, this may explain the pro- and anti-apoptotic properties of Cd salts at different concentrations. Additional studies after transfection of p53 gene into p53- epithelial cells or inactivation of p53 gene in p53+ cells may explain the mechanisms involved in MT induction and changes in apoptosis. (Supported by grants from CIHR and NSERC)

989 CADMIUM INDUCED APOPTOSIS IN HUMAN PROXIMAL TUBULE CELLS AND ITS DERIVED CELL LINE, HK-2.

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Cadmium (Cd^{+2}), a nephrotoxin can cause acute and chronic toxicity in man due to its slow accumulation in the proximal tubules of the kidney. Heavy metals have been known to cause cell death by necrosis or by apoptosis. The goal of this study was to determine if Cd^{+2}could induce apoptosis in the human proximal tubule (HPT) cells and its derived immortalized cell line, HK-2. For this purpose, HPT and HK-2 cells were exposed to lethal and sub-lethal concentrations of cadmium chloride for various time periods. Apoptosis was determined by observing the nuclear morphology with the nuclear binding dye, 4', 6-diamidino-2-phenylindole (DAPI), and internucleosomal DNA fragmentation. Continuous exposure of HPT cells to 27μM cadmium chloride for 12 to 60 hr caused cell death, with few cells showing the morphological changes typical for apoptosis and little DNA fragmentation. Exposure to 45μM cadmium chloride caused extensive cell death without any changes typical for apoptosis. HK-2 cells were more sensitive to Cd^{+2} and therefore lower doses of 4.5 and 9 μM Cd^{+2} were used which resulted in cell death with extensive chromatin condensation and DNA fragmentation. These results suggest that apoptosis is not the major mode of cell death elicited by Cd^{+2} in HPT cells and is concentration dependant, whereas HK-2 cells are more susceptible to death by apoptosis.

990 INDUCTION OF APOPTOSIS IN CELLS BY CADMIUM; QUANTITATIVE NEGATIVE CORRELATION BETWEEN BASAL OR INDUCED METALLOTHIONEIN CONCENTRATION AND APOPTOTIC RATE.

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Metallothionein (MT) often reduces the adverse effects of cadmium (Cd) but how it may alter Cd-induced apoptosis is unclear. Thus, the goal of this study was to define the role of MT in Cd-induced apoptosis using cell lines with widely varying sensitivity to Cd. Toxic effects of Cd on human hepatocellular carcinoma cell lines (HepG2 and PLC/PRF/5) were investigated and compared with Chang cells. These cells were cultured with 0, 5, 10, 20, 40, 80 and 120 μM of Cd for 3, 6, 12, and 24 h. Significant cytolethality was observed in HepG2 and PLC/PRF/5 cells in time-dependent and concentration-dependent manner with LC_{50} values of 24 μM and 13 μM, respectively. However, Chang cells were much less sensitive to Cd-induced cytotoxicity (LC_{50} = 64 μM). Apoptotic cell death occurring at cytolethal concentrations was demonstrated in all cell lines by DNA fragmentation with ELISA and confirmed with agarose gel electrophoresis. When MT was measured, there was a strong negative linear correlation between the rate of apoptosis induced by Cd in these cell lines and the basal levels of cellular MT concentration (r = - 0.996) or Cd-induced MT levels (r = - 0.975). Treating HepG2 cells with zinc (Zn) made the relatively sensitive HepG2 cell line resistant to Cd-induced apoptosis, likely due to Zn-induced MT. In fact, there was also a strong negative linear correlation (r = - 0.964) between the amount of Zn-induced MT in HepG2 cells and the rate of Cd-induced apoptosis. These findings revealed that Cd-induced apoptotic cell death in various cell lines was perturbed by basal or induced MT and a strong negative correlation exists between cellular MT content and the rate of apoptotic cell death induced by Cd.

991 CADMIUM INDUCES APOPTOSIS IN RAT KIDNEY PROXIMAL TUBULAR CELLS *VIA* THE MITOCHONDRIAL PATHWAY.

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Chronic cadmium (Cd) exposure causes renal proximal tubular cell damage and mitochondria appear to be the target organelle. The purpose of this study was to determine whether in these cells Cd-induced mitochondrial damage is responsible for apoptotic cell death. Normal rat kidney proximal tubular cells (NRK-52E) were exposed to 20 μM $CdCl_2$ for 5 h, followed by continuous culture in the absence of Cd for up to 12 h. Formation of reactive oxygen species was measured by hydrogen

peroxide accumulation and was found to be maximum 3 h after starting the Cd exposure. Cytochrome c release from the mitochondria to the cytosol peaked between 3 and 5 h. Caspase-3 activity was markedly elevated 12 h after stopping the Cd exposure and was higher in detached cells than in attached cells. Cytochrome c release was depressed upon inhibition of mitochondrial permeability transition with cyclosporin A along with the inhibition of phospholipase As_2 by aristocholic acid. The use of these inhibitors also decreased caspase-3 activity. Furthermore, both cytochrome c release and caspases-3 activity were depressed in cells over expressing Bcl-2. In conclusion, Cd-induced oxidative stress in rat kidney proximal tubular cell mitochondria appears to play a key role in apoptosis since generation of reactive oxygen species is followed by mitochondrial membrane permeability transition, cytochrome c release, and caspases-3 activation.

992 THE NITRIC OXIDE PRODRUG, V-PYRRO/NO, CONFERS RESISTANCE TO ARSENIC- AND CADMIUM-INDUCED CYTOTOXICITY AND APOPTOSIS IN CULTURED RAT LIVER CELLS.

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The liver is an important target tissue of both cadmium (Cd) and arsenic (As). The compound O2-vinyl 1-(pyrrolidin-1-yl)diazen-1-ium-1, 2 diolate (V-PYRRO/NO) is a liver-selective nitric oxide (NO) producing prodrug that is metabolized by hepatic P450 enzymes to release NO in hepatocytes. NO can compete for cellular binding sites with metals. Thus, we studied the effects of V-PYRRO/NO pretreatment on Cd (as $CdCl_2$) or As (as $NaAsO_2$) toxicity in cultured rat liver epithelial (TRL 1215) cells. Cells were pretreated with V-PYRRO/NO (at levels up to 1000 µM for up to 24 h) then exposed to Cd or As (for an additional 24 h) and cytotoxicity (by MTS assay) or apoptosis (by DNA fragmentation ELISA) was assessed. Cd was significantly less cytotoxic in V-PYRRO/NO (1000 µM) pretreated cells (LC_{50} = 6.1 ± 0.6 µM) compared to control cells (LC_{50} = 3.5 ± 0.4 µM). Likewise, the LC_{50} for As was 18.1 ± 1.4 µM in control cells and 27.3 ± 2.6 µM in V-PYRRO/NO pretreated cells. TRL 1215 cells acted upon the prodrug to release NO, producing nitrite levels (measured by Griess assay) in the extracellular media after 24 h of exposure to 500 or 1000 µM V-PYRRO/NO measured at 87.0 ± 4.2 and 324 ± 14.8 µM, respectively, compared to control levels of 7.70 ± 0.46 µM. Since both Cd and As are known to induce apoptosis, the effect of V-PYRRO/NO pretreatment on Cd- or As-induced apoptosis was studied. V-PYRRO/NO pretreatment (≥750 µM) markedly reduced apoptotic cell death induced by Cd (5 µM) or As (15 µM). Activation of the c-Jun N-terminal kinase (JNK) pathway can be critical to apoptotic cell death and pretreatment of cells with V-PYRRO/NO suppressed JNK activation after exposure to Cd or As. Thus, the prodrug, V-PYRRO/NO, protects against the adverse effects of Cd or As in rat liver cells in culture, including apoptosis and the concurrent activation of the JNK pathway, apparently through generation of NO. The role of NO in prevention of Cd or As toxicity deserves further study.

993 EFFECTS OF CADMIUM ON E-CADHERIN AND VE-CADHERIN/β-CATENIN MOUSE LUNG.

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Although it is well established that respiratory exposure to Cd2+ results in pulmonary edema and fibrosis, the specific mechanisms underlying these effects remain unknown. Recently, we have obtained evidence suggesting that Cd2+ produces these effects by interfering with the normal function of the Ca2+-dependent cell adhesion molecules E-cadherin and VE-cadherin. These molecules serve as important structural components of adherens junctions in alveolar epithelial cells and vascular endothelial cells respectively. In addition, the intracellular domains of the cadherins are linked to β-catenin, which under certain circumstances can be released from the junctional complexes and act as a regulator of gene expression. The objective of the present study was to characterize the effects of Cd2+ on the localization of E-cadherin, VE-cadherin and β-catenin in relation to the development of acute pulmonary injury. Male CF-1 mice were given either saline or CdCl2 (65 nmoles) *via* intratracheal administration instillation. After 24 hours, the lungs were subjected to bronchoalveolar lavage or weighed and processed for the immunohistochemical analyses and for the evaluation of cell viability with ethidium homodimer. The results showed that Cd2+ caused an increase in lung weight and in the protein content of the lavage fluid. These effects were accompanied by a pronounced decrease in the amount of E-cadherin in the epithelial cells of the alveoli and small bronchioles and of VE-cadherin in the vascular endothelial cells. In addition, the pattern of β-catenin labeling changed from co-localization with the cadherins at the plasma membrane to diffuse labeling throughout the cytoplasm. Additionally, a thin band of β-catenin labeling was observed at the cell nuclei. Assessment of cell membrane integrity with ethidium homodimer showed no evidence of severe injury or death of the alveolar epithelial or vascular endothelial cells. These findings indicate that the E-cadherin and VE-cadherin/β-catenin complexes may be important early targets of Cd2+ toxicity in the lung. Supported by NIH Grant # r01 ES06478.

994 EFFECTS OF P-GLYCOPROTEIN INHIBITORS ON CADMIUM ACCUMULATION IN CULTURED EPITHELIAL CELLS, OK, LLC-PK_1 AND CACO-2.

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The effect of P-glycoprotein (P-gp) inhibitors on cadmium (Cd) accumulation in cultured epithelial cells, OK, LLC-PK_1 and Caco-2, was investigated. These cells were pre-incubated for 30 min with a typical P-gp inhibitor such as verapamil, diltiazem, nifedipine, vinblastine or cyclosporin A before the incubation with 1 µM $^{109}CdCl_2$ for 15 min. Pre-incubation with each P-gp inhibitor increased Cd accumulation markedly in OK and LLC-PK_1 cells and marginally in Caco-2 cells. Similarly, treatment of these cells with UIC2, a P-gp monoclonal antibody, increased Cd accumulation significantly in OK and LLC-PK_1 cells and marginally in Caco-2 cells. Thus, the pre-treatment of cells with P-gp inhibitor appears to increase Cd accumulation as a result of the inhibition of Cd efflux *via* P-gp. To confirm Cd efflux *via* P-gp, Cd accumulation in P-gp-overexpressed cells (LLC-GA5-COL150 cells) was compared with that in parental LLC-PK_1 cells. LLC-GA5-COL150 cells accumulated less Cd than did LLC-PK_1 cells, and the effects of the P-gp inhibitors and UIC2 on Cd accumulation were profoundly observed in LLC-GA5-COL150 cells. These results suggest that P-gp in OK, LLC-PK_1 and Caco-2 cells appears to act as an efflux pump of Cd, decreasing cellular Cd accumulation.

995 HEAT SHOCK RESPONSE IN THE HUMAN UROTHELIAL CELL LINE, UROtsa, EXPOSED TO CADMIUM AND ARSENITE.

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The heat shock response of specific tissues to acute *versus* chronic exposure to toxic substances has not been addressed in the literature. We therefore wanted to determine if the heat shock response was a transient or persistent phenomena in UROtsa cells acutely and chronically exposed to chemical stress. UROsta cells are immortalized human urothelial cells that exhibit several features unique to normal urothelium. These cells were treated with sodium arsenite or cadmium chloride and mRNA and protein levels were detected by RT-PCR or western blots respectively. Acute exposure to 100 µM sodium arsenite or 53.4 µM cadmium chloride for 4 hours increased the levels of heat shock protein (hsp) 60, 70A, 70B and 70C mRNA and hsp 70 protein. There was no change in the level of hsp 27 mRNA and protein, and hsp 60 protein following acute exposure. Chronic exposure with 1, 5 or 9 µM sodium arsenite for 16 days had little effect on hsp 27, 60, 70A, 70B or 70 C mRNA levels, but hsp 70 protein was consistently present in cells treated with 5 and 9 µM sodium arsenite. Similarly, chronic exposure to various doses of cadmium chloride also demonstrated no obvious induction of hsp 27, 60, 70A or 70B genes. There was an increase in the levels of hsp 70C gene and hsp 70 protein in UROtsa cells chronically exposed to 5 and 9 µM cadmium, but the mRNA and protein levels decreased after 4 days with protein levels undetected after 7 days. These findings suggest that the heat shock response can be both transient and persistent, and that the duration of the response may be dependent upon the chemical treatment.

996 SIX-MONTH DRINKING WATER STUDY TO ESTABLISH A LOEL FOR CADMIUM TOXICITY.

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A lowest observed effect level (LOEL) associated with the oral ingestion of cadmium was investigated in a six-month drinking water study in rats. Male Sprague-Dawley rats were administered deionized drinking water containing 10, 25, and 50 ppm cadmium (as cadmium chloride) for durations of 30, 90, and 180 days. Signs of toxicity were evident at each timepoint, as evidenced by increased total urinary protein/24 hours and decreased body weight changes. At all three cadmium concentrations at days 90 and 180, urinary protein levels were greater than that of the control value. Among the three dose groups at days 30 and 180, body weight changes were decreased (65-85%) compared to control. Test animals adapted/compensated to the ingestion of cadmium over time for some parameters, and early increases in blood ALAD levels (156%-172% of control), and early decreases in

hematocrit (83-88% of control), urine production (15%-40% of control), and kidney ALAD (77% of control) were similar to control values within 180 days. Histopathological findings were unremarkable, other than increased inflammatory infiltrate between kidney tubules. This inflammation was observed at all timepoints and increased in severity with cadmium dose. At the 180-day timepoint, inflammation was most pronounced, and was characterized by an interstitial mononuclear cell infiltrate. A LOEL of 10 ppm was assigned and was based on decreases in hematocrit and urine volumes, increased proteinuria, and interstitial inflammation. This LOEL is currently being used in a metal/metalloid mixture study. [Supported by USEPA Star Grant R827161-01-0].

997 ANEMIA IN CHRONIC CADMIUM INTOXICATION IS INDUCED BY THE DESTRUCTION OF ERYTHROPOIETIN-PRODUCING CELLS IN KIDNEYS.

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Long term exposure to cadmium (Cd) induces renal tubular dysfunction and anemia. The two symptoms are thought to be closely related with each other, because plasma levels of one of the major hematopoietic factors, erythropoietin (Epo), which is produced mainly from the interstitial tissues of kidneys, do not increase in response to the anemia in human patients and animals with chronic Cd intoxication. Thus it is suggested that Cd induces anemia through disturbance of Epo production by targeting Epo-producing cells in the renal interstitial tissues. In order to investigate the local relationship between Cd-target cells and Epo-producing cells in kidneys, we performed *in situ* hybridization using Epo cDNA probe as well as hematoxilin-eosin staining on the renal tissue specimens of rats which were injected with Cd for 8 months. Epo mRNA was expressed in renal tubular cells, but those cells were destroyed and the interstitial tissues were replaced by fibrosis in Cd-intoxicated rats. This result indicates that anemia with hypoproduction of Epo observed in chronic Cd intoxication is induced by the destruction of Epo-producing cells in kidneys, i.e. renal tubular cells.

998 INTESTINAL ABSORPTION OF CADMIUM IS ASSOCIATED WITH DIVALENT METAL TRANSPORTER 1 IN RATS.

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The intestinal absorption of cadmium (Cd) increases when body iron (Fe) stores are depleted. The depletion of Fe up-regulates the expression of Divalent Metal Transporter 1 (DMT1), which is located in the apical membrane of enterocytes lining the small intestine. DMT1 has been shown to transport Fe and other divalent metal ions *in vitro*. However, it is not known whether DMT1 mediates the intestinal absorption of Cd. To investigate DMT1 involvement in Cd absorption, rats were fed a diet for 4 weeks either deficient in Fe (FeD-diet, 2-6 mg Fe/kg) or supplemented with Fe (FeS-diet, 120 mg Fe/kg), followed by a single oral administration of $^{109}CdCl_2$. Body Fe status, hemoglobin, and tissue Cd concentration were determined 48 hr after Cd administration. Also, DMT1 mRNA levels were quantified in duodenum, kidney, and liver by the branched DNA signal amplification method. Animals fed the FeD-diet exhibited a reduced body weight gain, depletion of body Fe, and Fe deficiency anemia. Tissue Cd concentration was significantly higher in FeD-diet fed rats than in FeS-diet fed rats especially in the duodenum. The amount of Cd retained in the body was 10-fold higher in rats fed the FeD-diet than in FeS-diet fed rats. DMT1 mRNA was highly expressed in duodenum and was 15-fold higher in the FeD-diet group. The levels of DMT1 mRNA were significantly lower in kidney and liver than in duodenum, but were 30 and 40 percent higher, respectively, in rats fed the FeD-diet than in rats fed the FeS-diet. These findings suggest that functional DMT1 protein is likely up-regulated in small intestine at the mRNA level by body iron depletion and increases Cd uptake from the gastrointestinal tract with subsequent transfer of Cd to the circulation and other body tissues. Furthermore, the data from this study may indicate that DMT1 is a non-specific metal transporter, which can transport not only Fe, but probably the toxic metal Cd as well. (Supported by NIH grants ES-01142 and ES-07079)

999 SUBACUTE HEMATOLOGICAL RESPONSES OF THE YUCATAN SAILFIN MOLLY *(POECILIA VELIFERA)* EXPOSED TO CADMIUM CHLORIDE.

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This study focuses on the subacute hematological responses of the Yucatan sailfin molly, *Poecilia velifera*, exposed to cadmium chloride. Previous studies in other teleosts and mammals have suggested that exposure to cadmium chloride results in a leucocytic response. Fish were exposed to nominal concentrations of 9, 5, 0.9, and 0.01 mg/L of cadmium chloride for seven days (n=100) in static exposure chambers. At the end of the exposure period, blood smears from surviving fish were taken *via* dorsal gill incision, fixed in methanol, and stained with Giemsa. Differential and total blood cell counts were determined and used to find the absolute blood cell count. Peripheral blood leucocytes, not previously characterized in this species of fish, were observed and described. Lymphocytes, large and small eosinophils, monocytes, and immature granulocytes were observed. Basophils and neutrophils were not observed in this species. Preliminary findings indicate a significant increase in the total leucocyte count as well as the absolute eosinophil count in fish exposed to cadmium chloride compared to the control group ($n < .02$). The potential use of the Yucatan sailfin molly as an environmental model for adverse hematological/immunological effects is discussed.

1000 ACTIVATION OF AP-1 DNA BINDING DURING MANGANESE TOXICITY IN PC-12 CELLS.

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Occupational and environmental exposure to manganese (Mn2+) is an increasing problem. It manifests neuronal degeneration characterized by dyskinesia resembling Parkinson's disease. The study was performed to test the hypotheses whether exposure to Mn2+ alters cellular physiology through intracellular signaling mechanism associated toxicity in neuronal cell lines. Using rat catecholaminergic rich pheochromocytoma (PC12) cells, various concentrations of Mn2+ (0-100 muM) were exposed and then cell viability was assessed by MTT assay. Exposure of Mn2+ to PC12 cells reduced cell viability in a dose dependant manner compared to the controls. Since transcription factors have been shown to play an essential role in oxidative stress related toxicity, we measured changes in DNA binding activity of activator protein-1 (AP-1) by electrophoretic mobility shift assay (EMSA) following Mn2+ exposure in PC12 cells. Mn2+ (0.1-10 muM) produced eight-fold-activation of AP-1 DNA binding activity. This significant increase was seen within 60 min period after Mn2+ exposure. Besides activating AP-1, Mn2+ also activated N terminal c-jun kinase suggesting that a JNK pathway is involved, which may in turn activate AP-1 *via* c-jun phosphorylation. This study clearly suggests that induction of signaling factor AP-1 during Mn2+ exposure may have a significant cytotoxic effect on neuronal function. This work was supported by RCMI grant RR03045).

1001 MANGANESE OXIDATION STATE AND CELLULAR TOXICITY.

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BACKGROUND: Chronic elevated manganese (Mn) exposure has been associated with neurological disorders. The introduction of the anti-knock agent MMT to gasoline has lead to concern about the potential for increased Mn exposures and neurotoxicity. The combustion products of MMT may increase exposure to a mixture of Mn oxidation states (Mn(II), Mn(III) and Mn(IV)), which may possess different relative toxicity's. However, little reliable data exist on the biological handling of Mn(II) *versus* Mn(III) from environmental exposures, or on the relative toxicity of these oxidation states in biological systems. This lack of data is due in part to the challenges associated with evaluating Mn oxidation state *in vivo*. Therefore, the goal of this research was to elucidate the relative toxicity of Mn(II) and Mn(III) in a cell model. Total 8-isoprostanes were measured as a marker of cellular toxicity in a dopaminergic cell line(PC12). Cells were exposed to Mn(II) or Mn(III) *via* culture medium (0 - 250uM). The validity of the Mn(II) and Mn(III) exposures were verified by EPR spectroscopy and ICP-MS. Since addition of the pro-oxidant Mn(III) directly to the cell culture system can damage cells *via* processes that may not be relevant to effects on intra-cellular processes, Mn(II) and Mn(III) were added to medium and incubated prior to exposing the cells. Cells were exposed for 18 hours. Results show that 8-isoprostane levels were significantly (~5-fold) increased following 250uM Mn(III) exposure, relative to controls. Exposures to Mn(II) at these levels did not result in measurable increases in 8-isoprostanes. These results indicate that Mn(III) is more toxic to cells than Mn(II) *via* a mechanism that involves increased oxidative stress and lipid peroxidation.

1002 THE EFFECT OF MANGANESE EXPOSURE ON IRON HOMEOSTASIS IN CULTURED PC-12 CELLS.

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Manganese (Mn) is a well-known neurotoxicant; however, its mechanism(s) of action remains elusive. We hypothesize that one factor contributing to Mn's neurotoxic effects is Mn-induced disruptions in intracellular iron (Fe) homeostasis as a

consequence of alterations to the post-transcriptional regulation of Fe responsive proteins. To investigate this hypothesis, undifferentiated PC-12 cells were cultured in 5% serum containing RPMI medium supplemented with 1-200 μM Mn as $MnCl_2$ for 24-72 hours. Intracellular Mn concentrations significantly increased over the culture period, reaching concentrations up to 50 times higher than that measured in control cells. This increase in intracellular Mn concentrations was correlated with changes in the intracellular Fe pool. In addition, the protein levels of the Fe-responsive proteins ferritin, transferrin receptor, and DMT-1 were also altered as a result of Mn exposure. The pattern in protein expression changes produced by exposure to Mn was similar to that observed for PC-12 cells cultured in the presence of the Fe-chelator, deferoxamine, our model for cellular Fe deficiency. These data demonstrate that Mn has the ability to disrupt not only the expression of Fe-responsive proteins, but also the intracellular availability of Fe, suggesting that Mn-induced changes in cellular Fe homeostasis may contribute to the neurotoxic effects of Mn.

1003 THE INFLUX OF MANGANESE, MANGANESE CITRATE AND MANGANESE TRANSFERRIN AT THE BLOOD-BRAIN BARRIER IS CARRIER-MEDIATED.

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Manganese (Mn) is required for normal brain function, yet at high levels it can cause neurotoxicity. Regulation of entry into and exit from the brain plays a critical role in determining whether Mn is at a safe or toxic concentration. In serum, Mn exists as the hydrated ion, in complexes with small molecular weight ligands and bound to proteins. The speciation of Mn may influence the kinetics of its movement across the blood-brain barrier (BBB). This study examined the brain entry of Mn^{2+}, Mn citrate and Mn transferrin (Tf), which respectively represent the hydrated, complexed and protein-bound fractions of serum Mn. The influx rates of each Mn species were determined in adult male rats using the *in situ* brain perfusion technique. These values were compared to predicted rates of diffusion across the BBB, which were determined from their octanol/aqueous partitioning coefficients and molecular weights. Brain influx transfer coefficients (K_{in} values) were determined in nine brain regions and the lateral ventricular choroid plexus from four or five perfusion durations per Mn species. K_{in} values from the nine brain regions were 5-13, 3-51 and 2-13 x 10^{-5} ml/s/g for Mn^{2+}, Mn citrate and Mn Tf, respectively. The choroid plexus K_{in} values exceeded those for all brain regions by >10 fold for each Mn species. These results suggest carrier-mediated brain influx of Mn^{2+}, Mn citrate and Mn Tf when compared to their predicted diffusion rates (1.5, 1.7 and 2.8 x 10^{-5} ml/s/g, respectively). K_{in} values were independent of a flow rate, determined at 10 and 20 ml/min. ^{55}Mn citrate inhibited ^{54}Mn citrate uptake, and $^{55}Mn^{2+}$ inhibited $^{54}Mn^{2+}$ uptake. The greater influx transfer coefficients for Mn citrate than Mn^{2+} suggest separate influx mechanisms. These results suggest Mn citrate may be a major manganese species entering the brain. Supported by Health Effects Institute, Research Agreement #99-10.

1004 BRAIN REGIONAL INFLUX OF ^{59}Fe: EEFFECT OF *IN VIVO* MANGANESE (Mn) EXPOSURE.

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Our recent studies indicate that chronic Mn exposure is associated with an increased Fe concentration in the cerebrospinal fluid (CSF), possibly due to the action of Mn on Fe regulatory mechanisms at the blood-brain barrier (BBB) and/or the blood-CSF barrier (BCB). This study was performed to explore the relationship between Mn exposure and the influx of ^{59}Fe to various brain regions. Rat brains were infused with Ringer solution, containing traces of ^{59}Fe with or without transferrin (Tf), for 15 minutes at 37 °C *via* the common carotid arteries. Brain regions, e.g., frontal cortex (FC), striatum (ST), hippocampus (HP), brain stem (BS), cerebellum (CB), and choroid plexus (CP) were then dissected, cerebral vasculature removed by a capillary depletion method, and radioactivity assayed by a gamma counter. In control rats, addition of Tf to the perfusate greatly decreased ^{59}Fe influxes to CSF, FC, CB, and BS, while the influxes to CP, ST, and HP were apparently increased. In the absence of perfusate Tf, Mn exposure significantly augmented the influx of ^{59}Fe to the CP, but not affect the influx to the rest regions examined. In the presence of Tf, however, the influxes of ^{59}Fe to most brain regions were unexpectedly reduced in Mn-exposed animals, except for a 3-fold increase into the CSF compared to the controls. These data suggest that aside from a TfR dependent transport, the CP may possess a non-TfR mediated pathway for Fe influx. Elevated CSF ^{59}Fe following Mn exposure may result from a redistribution of ^{59}Fe from brain parenchyma to the CSF, which may be subsequently removed by the CP. (Supported in part by Wellcome Foundation, NIEHS ES-08146 and ES-09089))

1005 MANGANESE (Mn) EXPOSURE ALTERS MITOCHONDRIAL MEMBRANE POTENTIAL IN HUMAN DOPAMINERGIC SH-SY5Y CELLS *IN VITRO.*

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Mn-induced cytotoxicity has been associated with mitochondrial disorders, including the inhibition of various enzymes in TCA cycle, disruption of electron transfer, and reduction in mitochondrial DNA (mtDNA). Since the electrical potential across the mitochondrial membrane reflects the integrity of mitochondrial function, we set out to test the hypothesis that Mn-initiated mitochondrial toxicity may ultimately lead to the alteration in mitochondrial membrane potential. Human dopaminergic SH-SY5Y cells were exposed to Mn as Mn chloride at the concentrations of 0.5-2 mM for 24 hr. The mitochondrial potential was determined by incubating the culture with Rhodamine 123, a mitochondrial specific dye, followed by Laser scanning confocal microtechnique (LCSM) analyses of fluorescence signal. Results showed that Mn treatment significantly decreased fluorescence signal by 26% as compared to controls, suggesting a diminished mitochondrial membrane potential after Mn treatment. The effects were Mn-concentration dependent and exposure-time related. Further study using flow cytometry revealed that Mn exposure increased the percentage of apoptotic cells to as high as 40% of controls. In addition, we found that Mn(III) appeared to be more cytotoxic than Mn(II) in induction of apoptosis at the concentration of 500 uM. These data confirmed that mitochondria may serve as the primary target for Mn cytotoxicity and that Mn-induced apoptosis may be a direct result of mitochondrial damage. (Support in part by National Natural Science Foundation of China Grant# 3000140 and Beijing Science Committee Grant# 9558102800).

1006 MANGANESE EXPOSURE INCREASES BLINK REFLEX EXCITABILITY IN SPRAGUE-DAWLEY RATS.

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Manganese (Mn) exposure may result in manganism, a neuromotor disorder with symptoms similar to Parkinson's disease (PD) and dystonia. One of the hallmark symptoms of PD is hyperreflexia. In both humans with PD, and in an animal model of PD, the trigeminal blink reflex is hyperexcitable. In alert rodents and humans, electrical stimulation of the supraorbital branch of the trigeminal nerve elicits a blink reflex as measured by orbicularis oculi EMG activity (OOemg). Blink reflex excitability can be measured by comparing the magnitude of reflex blinks evoked by pairs of identical, blink-evoking stimuli with short interstimulus intervals (ISI). This behavioral assay is known as the paired stimulus paradigm (PSP). We investigated the effects of 4 wks of Mn exposure on the trigeminal blink reflex in rats using the PSP. Under general anesthesia, male Sprague-Dawley rats were implanted with electrodes unilaterally for chronic OOemg recording, and a nerve cuff for stimulation of the supraorbital branch of the trigeminal nerve. Within one wk of the surgery, pre-Mn data was collected. Rats then received a 4.8 mg/kg dose of Mn, i.p./3 days a wk for four wks. Control rats received i.p. saline injections. Blink reflex data was collected once/wk throughout these 4 wks. Rats were killed and their brains saved for morphological and biochemical analysis. Within one wk of Mn exposure, Mn-exposed rats demonstrated a two-threefold increase in blink reflex excitability at ISIs from 50-300 ms, compared to pre-Mn responses. These increases were observed throughout the four wk testing period. Correlation of behavioral data with morphological and biochemical changes in basal ganglia was also undertaken. These data indicate that the blink reflex becomes hyperexcitable following Mn exposure. Measurement of blink reflex excitability may be a sensitive tool in the study of Mn neurotoxicity. Supported by Wilkes University. Type 1 and 2 Research Grants.

1007 INFLUENCE OF SUBCHRONIC MANGANESE SULFATE ON BEHAVIOR OF FEMALE RATS.

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The potential of manganese sulfate to produce neurotoxicity following ninety days of daily gavage was studied in female rats. The rats were gavaged with suspensions containing 0.1, 1.0, 10, 30, or 100 mg/kg manganese or the vehicle each day. The functional observational battery, the step-down task, and the sequential, matching-to-sample task were used in an initial screen for neurotoxicity. At the above doses manganese sulfate did not significantly affect the behavioral of the rats in the functional observational battery. The rats gavaged with 100 mg/kg manganese did show

less motor activity during the hour in the figure-8 maze, relative to rats gavaged with the vehicle. The gavaged doses of the trace metal salt did not significantly alter the performance of the rats on the step-down task. On the sequential, matching-to-sample task, rats were trained to retain a specific sequence of events over intervals ranging from 0.25 to 5 seconds. The accuracy of responding by the female rats at 30 mg/kg manganese decreased at longer time intervals. In summary, the ability of manganese at 30 mg/kg to decrease accuracy of responding at longer time intervals indicates that the trace metal at 30 mg/kg is impairing working memory on the sequential, matching-to-sample task. (Support by ATSDR grant U50/ATU398948)

1008 TRIGEMINAL UPTAKE AND CLEARANCE OF INHALED MANGANESE CHLORIDE IN RATS.

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Previously we have examined uptake and transport of inhaled manganese into the brain *via* the olfactory epithelium. We are now investigating the role of inhalation of environmental manganese in the development of Parkinson's disease. We have expanded our investigation of the transport pathways to include analysis of the trigeminal system. Proton Induced X-ray Emission (PIXE) is used to study the uptake, deposition, and clearance of soluble manganese chloride in the olfactory and trigeminal systems following nose-only inhalation exposures. Seventy Fisher 344 rats were exposed to manganese chloride, (2.2 ± .5 mg/m^3 of Mn, mass median aerodynamic diameter (MMAD) was 3.1 ± 1.4 microns). The OSHA ceiling Threshold Limit Value for Mn is 5 mg/m^3. Exposures were 6 hours/day for 10 days. Groups of rats (10 animals per group for dosed, 6 animals per group for control) were euthanized immediately (0), 7, 14, 30, 90, 180 and 360 days post-exposure. The brain, olfactory bulbs and trigeminal tissues are removed and cryosectioned into 10 micron thick sagittal sections. To date we have completed PIXE analysis of bilateral trigeminal tissues obtained from sacrifices at 0, 7, and 14 days. Mn dosed animals show statistically elevated levels of Mn in the trigeminal tissues at all three time points post exposure compared to vehicle controls. The maximum elevation seen in the 0 day animals decreases over successive time-points by a factor of 2, but has not reached background levels by the 14 day sacrifice. Identification of manganese within the trigeminal system raises the question of this pathway contributing to development of neurodegenerative diseases. This work is supported by NIEHS RO1-ES10766.

1009 RAT STRIATAL D-ASPARTATE UPTAKE IS REDUCED BY EXPOSURE TO MANGANESE.

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Excessive exposure to manganese can cause "manganism", a neurological disorder with extrapyramidal symptoms similar to Parkinson's Disease. The mechanism underlying manganese neurotoxicity is unknown and there is no effective treatment. The targets of manganese toxicity in the basal ganglia receive substantial glutamatergic input. Previous work has demonstrated excitotoxic lesions in the striatum, resulting from excessive stimulation of glutamate receptors, following manganese injection. This led us to hypothesize that manganese produces toxicity by altering glutamate neurotransmission in the basal ganglia. To test this hypothesis, male Sprague-Dawley rats were exposed to 1 or 10 mg of $MnCl_2.4H_20$/ml in drinking water for 60 days. Neurotransmitter uptake was determined in crude striatal synaptosomes prepared incubated at 37°C for 2.5 minutes. Uptake of D-aspartate, a nonmetabolizable glutamate analog, was reduced by 30% in rats exposed to 10 mg $MnCl_2$/ml, but was unchanged in those receiving 1 mg/ml. Dopamine and GABA uptake were not significantly altered by manganese exposure. The mechanism responsible for decreased aspartate uptake is unclear, but reduced cellular energy levels or transporter expression are likely mechanisms. The data suggest that manganese exposure causes neurotoxicity by decreasing excitatory amino acid uptake which may lead to excitotoxicity.

1010 PATHOLOGICAL CHANGES OF CHOROID PLEXUS FOLLOWING EXPOSURE TO LEAD AND CADMIUM IN RABBITS.

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Choroid plexus (CP) has been shown to sequester toxic metals such as lead (Pb) and cadmium (Cd). This study was performed to investigate morphological alterations in the CP following metal exposure. Rabbits received a single ip injection of 5, 25 and 50 mg Pb/kg or iv injection of 1, 2 and 4 mg Cd/kg. CP, cerebral cortex, CSF, and blood were collected at 4 and 24 h after dose administration for various assays and CP tissues fixed for pathological evaluation by light and electron microscopy. Concentrations of Pb and Cd were determined by AAS. Levels of glutathione (GSH) and total sulfhydryl (SH) groups were also assayed. Following exposure to Pb or Cd, the concentrations of both metals were significantly higher in the CP than those in the CSF, blood, and cerebral cortex. Under the light microscope, both metals caused vacuolation and granular degeneration in choroidal epithelia. By TEM, Pb treatment evidently altered morphology of the epithelial layer, characterized by increased numbers of lysosomes and swelling of mitochondria. Cd exposure produced abnormal choroidal convex luminal profiles, such as the loss in microvilli, increased cytoplasmic vacuolation, elevated numbers of lysosomes, dilated rough endoplasmic reticulum, and widened gaps between adjacent cells. While Pb injection increased GSH concentration in the CP, Cd exposure did not affect GSH status in the CP. The concentrations of total SH groups in tissues after Pb exposure were not different from those of controls. However, the total SH level of the CP in Cd-treated group was significantly lower than that of controls. These results suggest that sequestration of Pb or Cd in the CP can alter the morphology of the CP and that binding of toxic metals to GSH or tissue SH groups may underlie metal accumulation in this tissue. (Supported by National Natural Science Foundation of China Grant#39770642)

1011 INTERACTIONS BETWEEN TROPHOBLASTIC LEAD UPTAKE AND CALCIUM TRANSPORT MECHANISMS.

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in utero exposure to lead (Pb) is associated with neurobehavioral abnormalities in children and laboratory animals. During pregnancy, mechanisms favoring divalent calcium (Ca^{2+}) uptake by the fetus enhance mobilization of maternal Pb^{2+} stores and maternal intestinal absorption of Pb^{2+}. Previously, a lack of adequate Pb^{2+}-specific measurement techniques has prevented real time assessment of hypothesized interactions between trophoblastic Pb^{2+} uptake and Ca^{2+} transport mechanisms. A novel method, which measures Pb^{2+}-induced indo-1 fluorescence quench (I-1FQ), was adapted for the assessment of Pb^{2+} uptake into undifferentiated and differentiated Rcho-1 trophoblastic cells. A factorial experimental design allowed detection of significant interactions between treatments known to affect cellular uptake of Ca^{2+}. Statistical analyses were performed using ANOVA, and 3 to 5 replicates of each treatment protocol were performed. Pb^{2+} uptake increased with time following the addition of higher concentrations of Pb^{2+} (10 μM *versus* 1 μM) and, to a much lesser degree, with lower Ca^{2+} in solution (100 μM *versus* 1 mM). Activation of capacitative Ca^{2+} entry by 10 μM thapsigargin enhanced Pb^{2+} uptake into Rcho-1 cells, and this mechanism of Pb^{2+} influx was inhibited by 10 μM La^{3+}. Incubation with the midportion of parathyroid hormone-related peptide (PTHrP), an endocrine stimulus for Ca^{2+} uptake by Rcho-1 cells, increased the uptake of Pb^{2+} into undifferentiated Rcho-1 cells. The lower relative rate of I-1FQ in differentiated, as compared to undifferentiated, Rcho-1 cells was unexpected. This observation could be explained by the binding of Pb^{2+} to differentiation-associated Ca^{2+}-binding proteins instead of to indo-1. Uptake of Pb^{2+} by Rcho-1 cells was affected by interactions between differentiation status and other treatments known to impact cellular uptake of Ca^{2+}. The hypothesized facilitation of trophoblastic Pb^{2+} uptake by Ca^{2+} transport mechanisms was supported by these results.

1012 BLOCKAGE OF COPPER RELEASE BY LEAD IN ASTROGLIA *VIA* TARGETING HEAVY METAL BINDING DOMAIN OF ATP7A.

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Lead (Pb) is well known developmental neurotoxicant that may also be a risk factor for neurodegenerative disorders. Astroglia are the major site in brain for Pb accumulation. Astroglia are also the principal loci for other metals, such as copper (Cu) in Menkes disease, which offers a link for understanding some of the neurotoxic mechanisms of Pb. The central hypothesis of our work is that intracellular Pb exerts multiple neurotoxic effects by interfering with Cu transport and buffering mechanisms in astroglia. This hypothesis is based on compelling previous evidence from our laboratory that Cu accumulation in brain and in astroglial cultures is associated with Pb exposure, and that Cu efflux from C6 cells may be blocked by the binding of Pb to ATP7a (Menkes protein), a Cu-ATPase. We partially purified the heavy metal binding region (HMB) of ATP7a that was overexpressed in E. coli and showed that Pb has a stronger binding affinity than Cu for this construct. One molecule of HMB was found to chelate 11 Pb ions or 7 Cu ions, and Pb competitively bound HMB in the presence of Cu. These findings support the conclusion that Pb increases intracellular Cu levels in differentiated astroglia by blocking ex-

port *via* ATP7a. We also demonstrated the induction of several oxidative stress responses (induction of GRP78 and GRP94) in Pb-treated astroglia. The finding that oxidative stress occurred in Pb-exposed astroglia in culture, though Pb has no redox potential, indicates mediation by another ion, such as Cu.

1013 LEAD-INDUCED ALTERATIONS OF DOPAMINERGIC SIGNALING SYSTEMS IN PHEOCHROMOCYTOMA CELLS.

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Lead-induced alterations of the dopaminergic neurotransmitter system in specific brain regions appear to be closely associated with manifestations of behavioral changes. Moreover, alterations of the rate-limiting enzyme of the dopaminergic biosynthetic pathway, tyrosine hydroxylase (TH), have been associated with low-level lead exposure in various animal and cell models. However, the underlying biochemical and molecular events leading to these changes are obscure. Therefore, the objective of this study was to determine the effect of lead on the dopaminergic signaling system (i.e. Calcium Calmodulin dependent TH phosphorylation and activity) in pheochromocytoma (PC12) cells. PC12 cells are a particularly useful *in vitro* tool for this type of study because they synthesize, store and secrete appreciable amounts of dopamine. The results of this study revealed dose-dependent decreases of dopamine levels at .01, .05, .1, .5 and 1 μM lead exposure, followed by a dose-dependent increase to near control level at 10 μM lead exposure. CaMK II β protein expression exhibited significant increases from the control level at .05 and .5 μM of lead exposure, followed by significant decreases at 1 and 10 μM of lead exposure. There were overall decreases of tyrosine hydroxylase phosphorylation, and tyrosine hydroxylase activity with significant changes occurring at .5, 1, 5 and .05, 1, 5 μM lead exposures respectively. CaMK II activity exhibited biphasic changes with a significant increase at .05 and significant decreases at .5 and 1μM lead exposure. Further, this study revealed that 1μM lead decreased CaMK II β expression and inhibited CaMK II activity. The inhibition of CaMK II activity was associated with attenuated TH phosphorylation and TH activity, which subsequently lead to decreased dopamine levels. Hence, these findings suggest that lead-induced decreases in dopamine levels in PC12 cells may be due to its effects on the CaMK II dependent TH phosphorylation and TH activity. Supported by ATSDR, Cooperative Agreement #U50/ATU 398948 and NIEHS, Cooperative Agreement #ES 07290)

1014 ASSESSING THE ROLE OF THE DORSAL SUBICULUM/NUCLEUS ACCUMBENS PATHWAY IN PB-INDUCED CHANGES IN FIXED INTERVAL (FI) PERFORMANCE.

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Previous studies from this laboratory have demonstrated that Pb-induced increases in FI response rates are related to dopaminergic/glutamatergic alterations in nucleus accumbens (NAc). NAc integrates dopaminergic input from ventral tegmental area with glutamatergic inputs from hippocampus (Hip) and prefrontal cortex (PFC), areas considered critical in the regulation of cognitive and motivational behaviors. An asymmetrical disconnection procedure was used to better delineate the circuits involved in regulating FI performance. Rats trained on a FI-1 minute schedule of food reward were bilaterally implanted with guide cannulae in NAc core and the dorsal subiculum (DSub) area of Hip. Lidocaine was microinfused unilaterally in NAc core and on the contralateral side of DSub (disconnection), temporary inactivating the circuit, while leaving operative glutamatergic circuits connecting VSub with PFC and PFC with NAc. Lidocaine disconnection decreased FI responses rates, but did not affect post-reinforcement pause time. To test whether preferential AMPAergic activation of the operative DSub to PFC to NAc circuit could reproduce Pb-induced increases in FI response rates, doses of MK 801 (1.0-5.0 mg) or AMPA (0.03-0.3 mg) were infused in the non-lidocaine side of DSub in conjunction with the disconnection. This procedure likewise lowered rather than increased FI response rates. Collectively, these findings suggest that DSub/NAc pathway could play a role in increased FI rates, since rates fall after inactivation of this circuit. But preferential AMPA receptor activation of the DSub to PFC to NAc pathway may not be the operative mechanism, since it did not reproduce increased FI rates. Supported by ES01247, ES05017 and ES05903.

1015 LEAD-INDUCED AGGRESSIVE BEHAVIOR IN ADULT MICE.

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Lead is a heavy metal hazardous to both children and adults. Lead has no biological benefit to the body and exposure to the body can produce a range of toxic effects, especially in the nervous system. While some epidemiological studies have associated lead exposure with aggressive behavior, there has been little research in animal models. Experimental research on aggression in rodents has utilized mostly qualitative descriptions of the behavior. No method for quantitatively measuring the amount of aggressive behavior has been reported. We have developed a method for quantitative assessment of aggression in mice, and we report on the effects of lead exposure on aggressive behavior. Adult female and male CD-1 mice were treated with 0.25% or 0.5% lead acetate for 3 or 5 weeks. They were paired by gender for behavioral testing with or without isolation. Behaviors were videotaped for 5 minutes and evaluated using a scoring system. The system was validated in studies of isolation-induced behavior and studies of haloperidol as an anti-aggressive agent. Our results revealed quantitative differences in aggressive behavior between male and female mice exposed to lead suggesting involvement of the hormonal system. These results permit future investigations on the mechanism(s)underlying lead-induced aggressive behavior, which may be an important public health impact of lead exposure. Supported by: A Ronald McNair Fellowship to Lynn Calvin as an undergraduate student at Coppin State College

1016 METHYLMERCURY IMPAIRS NEURONAL DIFFERENTIATION BY ALTERING NEUROTROPHIN SIGNALING.

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In previous *in vivo* studies, we observed that developmental exposure to CH_3Hg can alter neocortical morphology and neurotrophin signaling. Using primed PC12 cells as a model system for neuronal differentiation, we examined the hypothesis that the developmental effects of CH_3Hg may result from inhibition of neurotrophin signaling through the Trk receptor. Stimulation of PC12 cells with nerve growth factor (NGF) for 24 hr resulted in robust neurite outgrowth, which was inhibited by CH_3Hg in a concentration-dependent manner (EC_{50}=0.03 μM). Whole cell binding assays using ^{125}I-NGF revealed a single binding site with Kd of ~1 nM. Exposure of PC12 cells to CH_3Hg (0.001 - 3 μM) had no effects on NGF binding. Following NGF binding, TrkA receptor autophosphorylation peaked at 2.5 min of NGF stimulation and was sustained up to 60 min. Concurrent exposure to CH_3Hg for 2.5 min resulted in a concentration-dependent decrease in TrkA autophosphorylation, which was significant at 0.1 μM CH_3Hg (~50%). To determine whether inhibition of TrkA activation affected downstream signaling, activation of MAP kinase (ERK1/2) and Protein Kinase C (PKC) were examined. Similar to TrkA, NGF-stimulation resulted in a time-dependent activation of ERK1/2, which peaks at 5 min and was sustained up to 60 min. Concurrent exposure to CH_3Hg for 2.5 min resulted in a concentration-dependent decrease in ERK1/2 activation, which was significant at 0.1 and 0.3 μM (~75%). While CH_3Hg inhibited PKC activity for several recombinant PKC isoforms (δ, ε, ζ) and in PC12 cell homogenates, there was no effect on NGF-stimulated PKC activity in whole cells. To correlate the neurochemical and morphological effects, PC12 cells were exposed to specific inhibitors of TrkA (K252A) and ERK1/2 (U0126) at concentrations selected to produce 50-75% inhibition. Both K252A and U0126 significantly inhibited components of neurite outgrowth. These data suggest that the inhibition of neurite outgrowth by CH_3Hg may be related to a disruption of the NGF signaling through the TrkA receptor.

1017 USE OF P53 GENETICALLY ENGINEERED PC12 CELLS FOR TESTING CHRONIC METHYLMERCURY NEUROTOXICITY.

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Methylmercury is an environmental hazardous substance known to induce neurotoxic effects. Apoptosis is known to be one of the mechanisms by which methylmercury induces neural cell death. The expression of the p53 gene is often described to be implicated in the induction of apoptotic mediated cell death. A novel genetically engineered PC12 cell line was developed at ECVAM (Stingele *et al.*, 1999), which has a controlled expression of the p53 gene according to the media used. The present work was therefore undertaken to test whether p53 expression is implicated in chronic methylmercury toxicity. After application of NGF, to induce cell differentiation and acquisition of neuron-like characteristics, cells were subchronically treated for 10 days with 10^{-9} M to 10^{-6} M of methylmercury. Results were compared to previous experiments carried out on aggregating primary brain cell cultures. The use of this novel genetically engineered PC12 cell line in an integrated testing strategy for evaluating chronic neurotoxicity of xenobiotics is discussed. Stingele S., Coecke S., Nicotera P. & Balls M. (1999) EC Patent filed 27th of

August 1999 on Genetically engineered cell lines, and their uses, in particular for neurotoxicity testing, European Patent Office, Rijwijk, The Netherlands, Patent No. 99402139.2-2106/International No PCT/EP00/08223

1018 STIMULATION OF ARACHIDONIC ACID RELEASE BY METHYLMERCURY IN PRIMARY CULTURES OF ASTROCYTES AND NEURONS.

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Cytosolic phospholipase A2 (cPLA2) stimulates the production of arachidonic acid (AA) from the sn-2 position of membrane phospholipids, the former serving as a precursor for the synthesis of prostaglandins. cPLA2 is upregulated in ischemia, epilepsy, and Alzheimer's disease, as well as by the excitotoxic neurotransmitter, glutamate. Glutamate uptake transporter inhibition is a known consequence of AA-mediated reactive oxygen species generation. There has been no systematic study examining the effect of methylmercury (MeHg) on astrocytic cPLA2 activation and AA release, or on the overall significance of this pathway to MeHg-induced neurotoxicity. In the present study, primary cultures of astrocytes and neurons were loaded overnight with [3H]-AA to label phospholipids. The effect of MeHg on the activation of cPLA2 was ascertained by measuring the release of radioactive fatty acid [3H]-AA from astrocytes or neurons over 120 minutes (at 1, 5, 10, 30, 60, 120 min). MeHg (5.0 μM) induced a significant ($p<0.05$) increase in AA release from astrocytes at 10, 30, 60, and 120 min. A significant increase in astrocytic AA release was observed with 2.5 μM MeHg only at the 120 min time point. No increase in neuronal AA release was noted at any time point upon treatment with 2.5 μM MeHg. MeHg (5 μM) induced a significant ($p<0.05$) increase in AA release at 5, 10, 30, 60, and 120 minutes. Consistent with these assays, studies demonstrate increased cPLA2 mRNA expression in astrocytes exposed for 6 hours to MeHg (5.0 and 10.0 μM). These results indicate that MeHg causes a dose- and time-dependent increase in the hydrolysis and release of AA *via* activation of cPLA2, lending credence to the hypothesis that its neurotoxicity is mediated, at least in part, by an excitotoxic mechanism. (Supported by PHS grant NIEHS 07331).

1019 MULTIPLE FACTORS CONTRIBUTE TO THE METHYLMERCURY (MEHG)-INDUCED EARLY STIMULATORY EFFECTS ON SPONTANEOUS SYNAPTIC CURRENTS OF PURKINJE CELLS IN CEREBELLAR SLICES OF RAT.

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Bath application of 20-100 μM methylmercury (MeHg) initially stimulates and then suppresses the frequency of spontaneous excitatory and inhibitory synaptic currents recorded from Purkinje cells in rat cerebellar slices using whole cell patch-clamp recording techniques. To determine whether the early stimulatory effect is the result of MeHg-induced elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), which increases release of neurotransmitter, the effect of MeHg on the frequency of spontaneous synaptic currents were first examined in reduced extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$). Reducing $[Ca^{2+}]_o$ from 2 to 0.5 mM did not significantly affect MeHg-induced early increases in the frequency of spontaneous synaptic currents, suggesting that Ca^{2+} influx is not a major contributor to the MeHg-induced early stimulatory effects. It is possible that increased release of Ca^{2+} from the intracellular stores is involved. To test this, ruthenium red (RR), an inhibitor of mitochodrial Ca^{2+} transport, was used to prevent the MeHg-induced release of transmitter. Preincubation of cerebellar slices with 20 μM RR diminished, but did not prevent MeHg-induced stimulatory effects on spontaneous synaptic responses. Preincubation of cerebellar slices with the membrane-permeable Ca^{2+} chelator BAPTA AM (40 μM) also failed to prevent MeHg-induced increases in spontaneous synaptic responses, suggesting that increased $[Ca^{2+}]_i$ is not the only factor contributing to the early stimulatory effects. Pretreatment of cerebellar slices with 40 μM TPEN, which chelates zinc but not MeHg, significantly reduced MeHg-induced increases in the frequency of spontaneous currents with larger amplitudes, but not those with smaller amplitudes, suggesting that the intracellular zinc may also play a role in MeHg-induced early stimulatory effects. Thus, multiple factors may be involved in the early stimulatory effects of MeHg on spontaneous synaptic currents in cerebellar Purkinje cells. Supported by NIH grant ES03299.

1020 METHYLMERCURY-INDUCED BLOCK OF VOLTAGE-GATED CA^{2+} CHANNELS IN ACUTELY ISOLATED CEREBELLAR PURKINJE NEURONS OF RAT.

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Cerebellar granule cells are more susceptible to the toxicity of methylmercury (MeHg) than are Purkinje cells. Voltage-gated Ca^{2+} channels are a prominent target of MeHg. To determine if the presence of a unique type of Ca^{2+} channel, or a differential expression of Ca^{2+} channels, confers upon granule cells and Purkinje cells a differential sensitivity to MeHg, we examined the effects of MeHg on current of voltage-gated Ca^{2+} channels in acutely dissociated Purkinje neurons from 7-11 day-old rats, using whole-cell recording techniques. Ba^{2+} currents in Purkinje cells were blocked 18.9±2.4%, 27.0±3.6% and 56.4±1.7% by nimodipine (1.0 μM), ω-conotoxin-GVIA (1.0 μM) and ω-agatoxin-IVA (50 nM) after 3 min exposure, respectively. Exposure of Purkinje cells to MeHg (0.5-5 μM) reduced peak and end currents in both time- and concentration-dependent manner. At 0.5μM, the effect of MeHg was greater on the sustained current remaining at the end of a test pulse than on the peak current. MeHg accelerated the time course of inactivation of the current. At 1.0 and 5.0 μM, the degree of block by MeHg was approximately the same for both peak and sustained components of current. Whole cell currents were reduced in amplitude by 95% after 3 min exposure to 5.0 μM MeHg. The current-voltage relationship was not altered after 3 min exposure to MeHg. However, the steady-state inactivation relationship was shifted to more negative potentials. Wash with MeHg-free solution did not reverse effects of MeHg. Increasing the stimulation frequency from 0.1 Hz to 0.2 Hz did not accelerate the block of I_{Ba} by MeHg. Comparing these results with previous results from granule cells (Sirois and Atchison, Toxicol Appl Pharmacol, 167: 1-11, 2000), the sensitivity of Purkinje cell Ca^{2+} channels to MeHg was almost the same as that in granule cells. Thus, it is unlikely that the presence of a unique Ca^{2+} channel subtype or differential complement of Ca^{2+} channels renders granule cells more susceptible to neurotoxicity of MeHg. Supported by NIH grant ES03299.

1021 EFFECTS OF METHYLMERCURY EXPOSURE ON EXPRESSION OF EPHS AND EPHRINS IN EMBRYONAL CARCINOMA CELLS.

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Repulsive interactions mediated by EPH receptors and ephrin ligands guide many morphogenetic processes, including the establishment of proper topographical mapping of various pathways within the CNS. Methylmercury (MeHg), a potent environmental neurotoxicant, induces morphological changes in the developing brain indicative of path-finding errors. The effects of MeHg on EPHs and ephrins were examined in P19 embryonal carcinoma (EC) cells and in neurons derived from P19 cells by retinoic acid induction. Undifferentiated EC cells and day 5 neurons were exposed to 0.5μM MeHg in the cell culture media. Following 24-hour exposure, total RNA was isolated from undifferentiated P19s or day 6 neurons and RNAse protection assays were performed. Levels of EPH and ephrin mRNA were evaluated using the RiboQuant RNAse protection assay system with receptor and ligand probe template sets. Undifferentiated EC cells expressed only ephrins-B2 and A5 and this expression was abolished by MeHg treatment. MeHg exposure also decreased expression of ephrins-B2 and A5 in day 6 neurons compared to control, while expression of ephrins-B3, B1, A6, A4, A3, and A1 remained unchanged. EPHs A1 and B3 were the only receptors detected in undifferentiated cells. EPHA1 expression was enhanced in MeHg treated cells while EPHB3 expression was decreased. EPHA1, A2, A3 and A4 expression was decreased in MeHg treated day 6 neurons *versus* control, while the remaining receptors appeared to be unaffected. These data suggest that selective perturbation of repulsive guidance molecules may play a role in the brain dysmorphogenesis caused by MeHg. (Supported by ES-05022 and 07148).

1022 THE EFFECT OF GESTATIONAL MERCURY VAPOR EXPOSURE ON RAT BRAIN α-SYNUCLEIN EXPRESSION.

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Alpha-synuclein is a highly conserved protein that localizes to pre-synaptic terminals and is thought to play a role in neuronal plasticity. It is upregulated developmentally and continues to be expressed at high levels in the adult brain. Its presence in a number of neuronal (AD, PD, dementia with Lewy bodies) and glial (Multiple System Atrophy) pathologies has led to the naming of a class of specific neurodegenerative diseases known as synucleinopathies. Alpha-synuclein has been implicated in a number of possible neurodegenerative mechanisms ranging from oxidative stress to changes in neurotransmitter processing. In addition, the pesticides rotenone, dieldrin and paraquat have been reported to increase the rate of synuclein fibril formation *in vitro*, similar to those found in amyloid plaques of AD and Lewy bodies of PD. These factors make α-synuclein a likely participant in environmentally induced neurotoxicity. It is well accepted that exposure to methyl mercury chloride has critical consequences in the developing brain. Recently concern has been raised that exposure to mercury vapor may be similarly neurotoxic (Fredricksson et al., 1996). Pathological changes such as decreases in cortical layer widths, diminished cell size and number and the induction of gliosis (Barone et al.,

1998) as well as behavioral changes in somatosensory information processing have been reported. A study was designed to examine the consequences of gestational (GD 6-15) mercury vapor exposure (4 kg/m3) on neuroanatomical and neurochemical endpoints. Here we report on the consequences on α-synuclein protein expression 1 and 2 years post-dosing in cortical, striatal and cerebellar regions of the rat brain. Data do not support long-term changes in brain protein levels of α-synuclein after gestational mercury vapor exposure. However, this does not eliminate the possibility of early perturbations in the formation of synaptic connections that might result in long-term pathology. This abstract does not necessarily reflect USEPA policy.

1023 BEHAVIORAL EFFECTS OF EXPOSURE TO HG^{O} FROM DENTAL AMALGAM.

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Potential neurotoxicity from exposure to mercury vapor (Hg^{o})from dental amalgam fillings is a subject of public health debate. A urinary Hg (HgU) screen of 4, 750 dentists in Washington State yielded 2, 835 samples with a range of 0-59 and mean (sd) of 2.51 (3.01)μg/l HgU. Among 1, 488 eligible dentists, 200 dentists and 200 assistants were enrolled to be representative of Hg exposures in the population (DDS = 3.08 (2.00); DA = 1.96 (1.82) μg/l HgU). A repeated cross-sectional behavioral study was conducted six months apart at a study center. Sessions included an evaluation of symptoms (transient and persistent), mood, cognitive, motor, and peripheral nervous system function in relation to HgU, applying the Behavioral Evaluation for Epidemiology Studies (BEES) touch-screen test battery. Hold tests included Vocabulary, WRAT, and visual acuity. Nitrous oxide (mean ppm =1, 944 (1, 611) was measured in air. Multiple regression models found statistically significant ($p<.05$) effects were most pronounced for mood (Beck' Depression Index and the SCL90 (Beta =.38 and .20 respectively) and symptoms (Beta=.18), followed by motor function (Hand Steadiness (Beta=.19) and Finger Tapping (Beta=.18)). Selective adverse cognitive effects were found for Tracking and Trails A (both Beta =.18), Visual Reproductions and Symbol-digit (both Beta =.13), and Digit Span (Beta=.11). Visual Contrast, Smell, and Nerve Conduction Velocity (1/Lat mV) also achieved significance (all Beta =.14). No effects were found for Trails B, Pattern Recognition and Memory, Reaction Time, Vigilance, and Vibration Sensitivity. Models controlled for age, alcohol, medications, and glasses. Mean dental urinary Hg levels for the USA (~5.0 μg/l), Washington State (~3.0 μg/l), and the general population (~3-9 μg/l) are consistent with levels at which we have first observed significant CNS deficits (1-5 μg/l) justifying characterization of chronic exposure to determine a safe level.

1024 MITOCHONDRIAL MEDIATED APOPTOSIS IN A HUMAN NEUROBLASTOMA CELL LINE (SK-N-SH) BY THIMEROSAL: A PROAPOPTOTIC ROLE FOR NUCLEAR FACTOR KAPPA BINDING PROTEIN (NF κB).

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The objective of this study was to examine the pathway of cell death in SK-N-SH cells by thimerosal, an ethylmercury containing preservative used in pharmaceutical preparations such as vaccines. Inorganic and organic mercurials cause apoptosis in cultured neurons; however, the signaling pathways resulting in programmed cell death have not been reported. SK-N-SH cells were exposed to thimerosal (0-10 μM) for 24 hours and then assayed by DNA fragmentation analysis. Cells treated with 5-10 μM thimerosal exhibited 250-300 bp nucleosomal fragments. To determine if the death pathway was mitochondrial mediated, JC-1 fluorescence, cytochrome c and apoptosis inducing factor (AIF) levels were analyzed. A significant decrease in JC-1 fluorescence was noted within 2 hours of treatment. Cytochrome c and AIF were shown to leak from the mitochondria in a dose dependent manner. Caspase 9 cleavage occured within 4 hours of treatment followed by caspase 3 activation. Within 12 hours poly(ADP)ribose polymerase, a downstream target of caspase 3 was cleaved to form 85 and 64 kDa proteolytic fragments. These results suggest a mitochondrial mediated apoptotic pathway in SK-N-SH cells upon thimerosal exposure. Reactive oxygen species (ROS) which contribute to mitochondrial mediated apoptosis increase upon mercurial exposure. A dose dependent increase in 4-hydroxy-2-nonenal adducted proteins was observed, indicative of ROS formation and lipid peroxidation. Sustained levels of the redox sensitive transcription factor, NF κB, was observed with 10 μM thimerosal. However, manganese superoxide dismutase, a NF κB target gene and antioxidant known to attenuate mitochondrial mediated apoptosis, was not induced suggesting a proapoptotic role for the transcription factor. In summary, this study identifies NF κB as a potential pharmacological target for intervention of thimerosal mediated neurotoxicity.

1025 THIMEROSAL INDUCES MICROTUBULE DEPOLYMERIZATION IN DEVELOPING NEURONS.

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Thimerosal has been widely used in vaccines as an antiseptic and preservative. Recently, its use has come under scrutiny due to the potential neurotoxicity of its chief component, ethylmercury, but its exact mechanism of toxic action is unknown. Thimerosal acts as a sulfhydryl binding agent and increases intracellular calcium levels. Since the microtubule (MT) network is highly susceptible to perturbations by –SH agents and/or elevations of cytosolic calcium, disruption of MT-dependent functions may underlie the compound's toxicity. The effects of thimerosal and a known anti-MT agent, methylmercury (MeHg) on MTs of undifferentiated cells and neurons were compared using P19 embryonal carcinoma cells. EC cells differentiate into neurons following induction by retinoic acid (RA). EC cells and developing neurons were exposed to 0-4.5μM thimerosal or MeHg for 2-24 hrs, then fixed or allowed 22 hr recovery in control medium prior to staining with FITC-conjugated anti-α tubulin (clone DM1A) or neuron-specific βIII tubulin. MTs were examined by epifluorescence microscopy. Thimerosal induced robust disassembly of MTs in both undifferentiated cells and developing neurons. In EC cells, loss of MTs occurred at thimerosal concentrations as low as 0.1μM and MTs were completely lost at 1.5μM and above. In developing neurons, 1.5μM disassembled most perikaryal MTs and induced retraction of neuritic processes. At 2.5μM and above, MTs were completely absent and no neurites were observed. Both undifferentiated cells and developing neurons were less sensitive to MeHg-induced MT disassembly, with complete loss of MTs occurring at 2.5 and 3.5μM, respectively. Recovery of MTs in thimerosal-treated cells was incomplete even after 22 hrs and levels above 2.5μM appeared lethal to most cells. In contrast, substantial MT recovery was observed in MeHg cells up to 2.5μM. Data indicate that thimerosal is more toxic than MeHg to MTs in undifferentiated and neuronal cells. Effects on MTs may play a significant role in the toxicity of thimerosal. (Supported by NJ Governor's Council on Autism and ES 05022)

1026 RESPONSE OF EPENDYMAL STEM CELLS TO NEUROTOXIC DAMAGE.

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Numerous studies have confirmed the presence of neural stem cells capable of forming neurons within the adult brain. These cells have been isolated from several cortical regions, including hippocampus and the ependymal/subventricular zones. To test the hypothesis that neurotoxic injury may signal proliferation and migration of ependymal stem cell progeny as part of a repair response in the brain, C3H mice were unilaterally injected icv with the lipophilic fluorescent dye DiI and given the hippocampal neurotoxicant trimethyltin (TMT; 2.5 mg/kg, ip). Bromodeoxyuridine (BrdU) was administered daily (ip) to label proliferating cells. Brain sections from mice sacrificed 3, 7, and 14 days post-TMT were examined by fluorescence microscopy. DiI labeling appeared confined to ependymal cells and their progeny. At 7 days post-treatment, DiI-labeled cells were observed migrating from the subventricular zone toward the hippocampus and by day 14 DiI-tagged cells were observed within the inner hilus of the dentate gyrus. The ependymal lining adjacent to migrating DiI-labeled cells often exhibited "pitting" and decreased fluorescence implicating the ependymal layer as their point of origin. NeuN+/BrdU+ cells were visible in the dentate gyrus and subependymal zone 3 days after TMT treatment; however, at this time these cells were DiI-. A marked astroglial response consisting of GFAP+/BrdU- cells was seen in the hippocampus throughout the study. These results suggest two phases of neuronal replenishment occur following neurotoxic injury. The rapid first phase involves cells resident within the dentate while the second, slower phase involves activation and recruitment of cells originating in the ependymal layer. The relative contribution of the two phases to the final repair of damaged neural tissues remains to be determined. (ES-10153, ES-07148, ES-05022)

1027 INHIBITION OF NEURONAL OUTGROWTH IN HIPPOCAMPAL NEURONS IN CULTURE BY TRIMETHYLTIN.

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Trimethyltin, (TMT) is an organotin neurotoxicant, which specifically affects pyramidal neurons in the hippocampus. The exact mechanism of toxicity of TMT is as yet unknown. The objective of this project was to examine the effect of TMT on

neural outgrowth, a developmental process, in hippocampal pyramidal cells in primary culture. Hippocampal neurons go through a highly stereotyped developmental sequence leading to outgrowth of axons. Stage 1 involves generalized motility, Stage 2 involves the outgrowth of short minor processes and Stage 3 involves the outgrowth of a long axon. Hippocampal cells isolated from 18 day old rat embryos were exposed *in vitro* for 24 hrs to 0.125μM, 0.25μM, 0.5μM, 1μM, or 2μM TMT, or TMT-free solutions as a control. TMT was subsequently removed, and photos of developing cells were taken at 24, 48 and 72 hrs after completing treatment. The number of cells in each stage was counted. TMT-free solutions did not compromise the outgrowth of hippocampal neurons at 24, 48 or 72 hours. By 72 hours, ~ 65% of the cells had progressed to Stage 3, and less than 20% remained in Stage 1. In contrast, TMT had a concentration-dependent action to suppress neuronal process outgrowth of hippocampal neurons. At low concentrations (0.125-0.5 μM) TMT moderately slowed the progression of neurons from stage 1 to subsequent stages. By 72 hrs, ~ 25-35% of the cells had progressed to Stage 3. However, at 1 and 2μM TMT, the slowing of growth progression was more prominent, and more long-lasting. Less than 25% of the cells progressed to Stage 3 under these conditions, and 40-50% of the cells remained in Stage 1. Thus acute exposure to TMT can affect the processes of neuronal growth and differentiation in hippocampal neurons in culture. Supported by NIH grant R03-ES10176.

1028 AUTOANTIBODIES TO NERVOUS SYSTEM PROTEINS FOLLOWING TRIMETHYL TIN (TMT) EXPOSURE: A COMPARISON OF ELISA AND WESTERN-BLOT ANALYSIS.

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Previous studies in both human and animals have demonstrated the presence of serum autoantibodies to neurotypic [e.g., neurofilament triplet (NF)] and gliotypic proteins [myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP)] following exposure to some heavy metals, solvents, or pesticides. TMT has been used as a denervation tool to validate the enhanced expression of GFAP as a biomarker of astrogliosis resulting from neuronal damage and cell death. In particular, TMT targets hippocampal pyramidal neurons in CA3 and CA1. In the present study TMT was used to assess the detection of the serum polyclonal IgG responses against NFs, measured by ELISA, as a peripheral marker of neurotoxicity. Western-blots, using purified NF proteins, or hippocampal homogenates, were used to confirm ELISA. Male Long-Evans rats (45 days of age) were administered either TMT (8mg/kg; sc; n=16) or an equal volume of sterile 0.9% saline (n=16). At 3 weeks post-administration, serum was collected, and rats were sacrificed for the collection of brains. The polyclonal IgG response to NF68, NF160, and NF200, using ELISA, showed detectable titers of IgG autoantibodies to NFs in sera from TMT-exposed rats, only. Anti-NF68 titers were highest compared to NF160, or NF200. Immunostaining of Western-blots using HRP-conjugated anti-rat IgG confirmed the presence of antibodies, against purified proteins or hippocampal homogenates, in sera of rats exposed to TMT. This corresponded to the molecular weights of NF 68, NF160, and NF200. Immunoblot confirmed the use of the ELISA as a means of detecting the autoantibody response. This study suggests that the detection of autoantibodies to neurotypic proteins, using ELISA, may be used to indicate chemical neurotoxicity. (Supported by NIH HD36965 and USDE P217A990192).

1029 ZINC-INDUCED MODULATION OF CHOLINE TRANSPORT IN CULTURED CHOROID PLEXUS.

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Removal of choline from cerebrospinal fluid (CSF) by choroid plexus is a potentially critical regulatory point in central cholinergic homeostasis. Modulation of the transport pathway by chemical stress may alter choline availability in the CNS. We examined the potential effects of zinc on choline removal from CSF in primary cultures of neonatal rat choroid plexus epithelium. Cultures were exposed to 10 μM $ZnCl_2$ in serum-free medium for 1.5 h (37°C). After 0-12 h recovery in Zn-free medium/5% NuSerum ± severe heat shock (SHS, 45°C, 1 h) ventricular uptake of 10 μM 3H-choline ± 750 μM hemicholinium-3 was assayed in artificial CSF (30 min, 37°C). LDH release was assayed to evaluate cytotoxicity. HSP70 accumulation was analyzed by immunoblot and normalized to actin band intensity. To assess cytoskeleton integrity and general cell morphology, actin was probed with TRITC-labeled phalloidin and viewed by fluorescence microscopy. After Zn exposure choline uptake was comparable to that by untreated controls with LDH release increasing 12%. However, uptake was stimulated 15% after 1-h recovery. Stimulation persisted through 12-h recovery, at which time uptake was enhanced 50%. Thermotolerance of transport was also enhanced with recovery. Without Zn/recovery preconditioning, SHS increased LDH release <5% but reduced choline uptake 50%. However, after Zn exposure, SHS reduced uptake by only 25%. In cultures subjected to SHS after 1-6 h recovery, uptake was comparable to that by non-SHS controls. After 12-h recovery, SHS reduced uptake by 20%. Thus complete thermoprotection was transient. Zn exposure also induced HSP70 accumulation, with levels peaking 3-6 h post Zn exposure and declining thereafter. While overall cell morphology was maintained, Zn-exposure induced migration and localization of actin filaments to the cell periphery. These data suggest that zinc may induce latent stimulation and thermoprotection of choroid plexus removal of choline from CSF, possibly correlated with induction of HSP70 and the actin cytoskeletal reorganization. ES10439; NS39452

1030 TRANSPORT OF ZINC IN THE OLFACTORY PATHWAYS OF RATS.

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The olfactory route provides a pathway for metals to the brain, thus allowing them to circumvent the blood-brain barrier. In the present study zinc-65 was instilled intranasally in rats and the disposition of the metal in the olfactory system was examined. Autoradiography with tape-sections showed a transport of the metal to the terminals of the primary olfactory neurons in the glomeruli of the olfactory bulbs, followed by a passage across the synapses to the interior of the bulbs. Cell fractionations showed that the zinc in the olfactory epithelium and the olfactory bulbs was present both in the cytosol and in association with various particulate cell constituents. Gel filtrations of the cytosolic fractions on a Superdex 30 column showed that the zinc was eluted in the void volume (MW >10, 000 Da). Intranasal pretreatment of rats with cadmium, to induce metallothionein, did not affect the subcellular distribution of zinc. Our results show that zinc is transported along the olfactory pathway. The data indicate that during this process the metal is bound to both particulate cellular constituents and cytosolic components, the latter having a high molecular weight.

1031 ENVIRONMENTAL RISK FACTORS FOR ALZHEIMER'S DISEASE.

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Alzheimer's disease (AD) is a progressive, neurodegenerative disorder, characterized, in part, by the extra-neuronal accumulation of beta-amyloid peptide. This peptide is a cleavage product of the amyloid precursor protein (APP). Aggregation of this peptide is suspected to lead to neuronal damage and cell loss. The factors that result in the aggregation of this peptide are not known, however, it is possible that exposure to environmental agents could promote such aggregation. Previous studies have alluded to the presence of Zn and potentially other metals in the amyloid plaques (Bush et al., JBC, 2000). In an attempt to identify environmental contributors to such a process, we screened a variety of metals and tested their ability to enhance beta-amyloid aggregation. Synthetic Abeta (1-40) peptide was obtained and used in an assay to determine its aggregation in the presence of a series of metals. The assay involved measuring Abeta aggregation by examining the fluorescence spectra that are produced at 482nm only when the aggregated peptide binds to the Thioflavin T probe. Initially the aggregation of the peptide was studied by varying the pH (pH 5-8) of the buffer. Subsequently, various metals were tested for their ability to enhance aggregation beyond that produced by pH alone. The metals studied were Zn, Pb, Mn, Ba. Pb and Mn promoted aggregation at pH 5-6, at a concentration of 10μM, while both Zn and Ba had minimal effects at this concentration. These preliminary results suggest that environmental metals may contribute to amyloidogenesis, however, further *in vivo* and *in vitro* studies are needed to confirm such an outcome.

1032 INHIBITION OF HUMAN SQUALENE MONOOXYGENASE BY SELENIUM COMPOUNDS.

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Excessive selenium exposure leads to a variety of toxicities in animals and in man. However, the chemical species of selenium and the molecular targets that mediate its toxicity are not well characterized. Methylated metabolites of tellurium, which is located directly below selenium on the Periodic Table, are potent inhibitors of squalene monooxygenase, the second enzyme in the committed pathway for cholesterol biosynthesis, and lead to a peripheral demyelinating neuropathy similar to that seen in selenosis. To evaluate the toxicity of selenium and its methylated metabolites, we

examined the ability of selenite and three methylselenium compounds, methylselenol, dimethylselenide, and trimethylselenonium iodide, to inhibit purified recombinant human squalene monooxygenase. Selenite proved to be the most potent inhibitor of this enzyme, with an IC_{50} value of 37 μM; methylselenol (95 μM) and dimethylselenide (680 μM) were less effective inhibitors, and inhibition by trimethylselenonium iodide was evident only at concentrations above 3 mM. The inhibition by methylselenol and selenite was slow and irreversible, but activity could be protected and restored by the addition of monothiols (glutathione, β-mercaptoethanol), indicating that the selenium compounds were reacting with sulfhydryl groups on the protein. Unexpectedly, the inhibition by selenite was significantly enhanced by dithiols (dimercaptopropanol, dithiothreitol), indicating that a more toxic species, possibly selenide, was formed in the presence of these dithiol reductants. These results raise the possibility that inhibition of squalene monooxygenase by selenite, selenide, and methylselenol may contribute to the neurotoxicity of selenium. Supported by a grant from the American Heart Association.

1033 INCREASED EXPRESSION OF PERIPHERAL BENZODIAZEPINE RECEPTORS IN THE MOUSE BRAIN FOLLOWING CUPRIZONE-INDUCED DEMYELINATION.

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The peripheral benzodiazepine receptor (PBR) has been used as a sensitive marker of gliosis and inflammation associated with neurotoxicity. Previous reports have suggested that PBR expression following brain injury is specific to areas expressing activated glial cells. Cuprizone, a copper chelator, has been used as a neurotoxicant to induce brain demyelination in mice. In the present study, we used this model in order to broaden the application of PBR as a biomarker of neurotoxicity, and to validate the relationship between PBR expression and glial cells. Adult male C57BL/6J mice were continuously fed a 0.2% (w/w) cuprizone-mixed powder diet and sacrificed at 2 and 4 weeks of treatment. [3H]-(R)-PK11195 autoradiography was performed to quantitatively measure PBR levels in anatomical regions. Increased expression of PBR was present in most white matter and in some gray matter regions of the cuprizone-exposed brain. Increased PBR levels were a function of the duration of cuprizone treatment. Consistent with previous studies on cuprizone neuropathology, significant ($p<0.05$) increases in PBR levels at 2 weeks of treatment were noted in hippocampus (117%), frontal cortex (114%), temporal cortex (106%), and entorhinal cortex (98%) relative to controls. A greater number of regions and higher levels of PBR binding ($p<0.01$) were measured at 4 weeks of treatment. These included: cerebellar peduncles (2907%), corpus collosum (2370%), intermediate white layer superior colliculus (1090%), globus pallidus (917%), thalamus (825%), cerebellar deep nuclei (475%), striatum (389%), hippocampus (256%), and different areas of the cerebral cortex (226-332%). Present studies are assessing the glial cell type contributing to the increased levels of PBR in this model of demyelination. These studies support the notion that PBR can be a useful marker to visualize and measure neuropathological changes in brain following treatments that result in demyelination. [Supported by grant # ES07062 to TRG]

1034 URANYL ACETATE-INDUCED SENSORIMOTOR DEFICIT AND INCREASED NITRIC OXIDE GENERATION IN THE CENTRAL NERVOUS SYSTEM OF RATS.

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In the present studies, we investigated the effects of uranyl acetate as a surrogate for DU on sensorimotor behavior, generation of nitric oxide and the central cholinergic system of rats. Male Sprague-Dawley rats were treated with i.m injection of 0.1, and 1mg/kg uranyl acetate in water, daily for 7 days. The animals were maintained treatment free for an additional observation period of 30 days. On day 30 following the cessation of the treatment, the sensorimotor functions were evaluated using a battery of tests that included measurements of postural reflexes, limb placing, grip time, beam walking and incline plane performance. The levels of nitric oxide as marker for increased oxidative stress, and the integrity of the cholinergic as reflected in acetylcholinesterase activity and m2 muscarinic acetylcholine receptors ligand binding, in brain regions were determined. The data from behavioral observations showed that there was a dose-related deficit in incline plane performance. Both doses significantly reduced grip time. Similarly, both beam walk score and beam walk times were impaired at both doses as compared with the controls. A significant increase in nitric oxide was seen at 0.1 mg/kg dose in cortex and midbrain, whereas brainstem and cerebellum showed a decrease at both the doses. There was a significant increase in acetylcholinesterase activity in the cortex of the animals treated with1 mg/kg uranyl acetate, but not in other brain regions. Ligand binding densities for the m2 muscarinic receptor did not show any change. These results show that low-dose, multiple exposure to uranyl acetate, causes prolonged neurobehavioral deficits after the initial exposure has ceased, and suggest that exposure with uranium/DU to may cause long-term neurological deficits.

1035 MOLECULAR MECHANISMS OF CHEMICAL TERATOGENESIS.

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Although over 50 years have passed since the thalidomide tragedy left thousands of infants born with severe malformations, only recently have a variety of biochemical and molecular biological approaches begun to reveal the underlying mechanisms and risk factors for chemical teratogenesis. This symposium provides a timely update of four approaches which, while diverse in technical repertoire and mechanistic thrust, all focus upon teratological mechanisms in the conceptus, providing both corroborating results and novel insights. First are the roles of reactive oxygen species-mediated signal transduction and oxidative macromolecular damage in teratogenesis, and the contributions of embryonic antioxidative enzymes and DNA repair in modulating teratologic risk. The second speaker focuses upon embryonic cellular thiols and redox-regulated developmental processes in sensitive and resistant species. The third approach investigates the teratological significance of alterations in gene expression and signal transduction pathways regulating conceptal cell cycle checkpoint activation, DNA repair and apoptosis. The last speaker focuses on the signaling pathways modulating apoptotic cell death in the developing embryo, and how their perturbation by xenobiotics may lead to teratogenesis.

1036 REACTIVE OXYGEN SPECIES (ROS) AND OXIDATIVE DAMAGE IN TERATOGENESIS.

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Using xenobiotics (phenytoin, thalidomide, BENZO(A)PYRENE (B[a]P)) and gamma irradiation, we examined the teratologic relevance of embryonic bioactivation by prostaglandin H synthases (PHSs), ROS-mediated oxidative macromolecular target damage and signal transduction, and putative DNA repair in mouse, rat and rabbit models. The xenobiotics were bioactivated by purified PHS to free radical intermediates. PHS-2 expression was high in mouse embryos during organogenesis, xenobiotic embryopathy was reduced by PHS inhibitors and in PHS-1 and PHS-2 knockout mice. Xenobiotic bioactivation, DNA/protein oxidation and embryotoxicity occurred in embryo culture. *in vivo* embryonic DNA oxidation was reduced to baseline within 24 hr, indicating active DNA repair. Treatment with antioxidative enzymes increased embryonic antioxidative activity, blocked DNA oxidation and inhibited teratogenicity, as did free radical trapping agents and antioxidants. Thalidomide embryonic DNA damage occurred only in susceptible (rabbit) but not resistant (rodents) species. Hydroxyl radicals were formed *in vivo*, and teratogenicity increased either with mutant mice deficient in the antioxidative enzyme glucose-6-phosphate dehydrogenase, GSH depletion, or inhibition of GSH peroxidase or GSH reductase. Nitric oxide synthases (NOSs) likely contribute to ROS-mediated teratogenesis, since iNOS knockout mice were partially protected. Inhibition of Ras reduced embryotoxicity, implicating ROS-mediated signal transduction. DNA may be a teratologically important target for oxidative lesions, since Atm and p53 knockout mice were more susceptible to teratogenesis, in some cases independent of apoptosis. These studies suggest that embryonic processes regulating the balance of ROS signaling, oxidative DNA damage and repair may be important determinants of teratologic risk. (Support: CIHR)

1037 REDOX-SENSITIVE MISREGULATION OF LIMB OUTGROWTH BY THALIDOMIDE.

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The ability of thalidomide to become bioactivated and generate free radicals and/or reactive oxygen species has been shown in several developmental and disease models. Differences in the glutathione (GSH)/redox status and regulation in limb cells

from resistant (rat) and sensitive (rabbit) species suggest that redox regulation may be important in mechanisms of thalidomide-induced limb reduction defects. Exposure of whole animals, tissues and cultured limb cells show that the rabbit limb has lower overall levels of GSH and cysteine and cannot maintain a reducing nuclear redox environment when exposed to thalidomide. Limb outgrowth was regulated by NF-kB, a redox-sensitive transcription factor. No differences exist between sensitive and resistant species in terms of the ability of thalidomide to activate NF-kB in the cytosol and translocate it to the nucleus. The rabbit, however, is unable to maintain a reducing nuclear environment, not allowing NF-kB to bind its promoter and initiate limb outgrowth. Whole mount *in situ* hybridization of rabbit embryos exposed to thalidomide *in utero* with probes directed to fgf-10, twist and fgf-8 show that thalidomide blocks or attenuates the expression of these limb outgrowth genes as a result of the inability of NF-kB to bind its nuclear promoter. NF-kB-mediated gene expression and limb outgrowth is restored by the addition of phenylbutylnitrone, a free radical trap. These processes are not affected in the resistant rat. Our studies show how environmental factors and chemicals can interfere with endogenous gene expression to result in developmental disease and malformation. Selective redox-misregulation of gene expression may be a critical factor in the mechanisms of developmental abnormalities produced by thalidomide and other chemicals capable of eliciting oxidative stress. (Support: OVPR-University. of Michigan)

1038 HOW DOES THE CONCEPTUS RESPOND TO GENOTOXIC STRESS?

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Many teratogens, either directly or *via* reactive oxygen species, increase DNA damage in the conceptus. Mammalian cells have evolved complex genotoxic stress responses to detect DNA damage and maintain genomic integrity, including DNA repair, cell cycle arrest, and apoptosis. We hypothesize that the ability of the conceptus to respond to genotoxic stress is a determinant of the outcome of teratogen exposure. Our first goal was to elucidate the expression profile of DNA repair/stress response genes during organogenesis in the rat. Second, we determined the consequences of exposure to a DNA damaging teratogen, 4-hydroperoxycyclophosphamide (4-OOHCPA). Gestational day (GD)10-12 rat conceptuses were analysed with the aRNA technique. Transcripts for 17 nucleotide excision repair (NER) genes, the major mammalian DNA repair pathway, were expressed at low levels on GD10, except for XPD, XPE and PCNA. There were stage dependent increases in XPB expression in yolk sac and in hHR23B, XPB, XPE, ERCC1, and DNA polymerase epsilon expression in the embryo from GD10-12. Whereas 4-OOHCPA induced malformations, it did not affect NER transcript levels. Except for UNG, APNG, PMS1, and RAD54, genes in the base excision repair (BER), mismatch repair (MMR) and recombination repair (RCR) pathways were not detected or expressed at low levels on GD10. Interestingly, UNG transcripts (BER) increased from GD11-12 by over 400%. Unlike the NER genes, 4-OOHCPA down-regulated transcripts for most BER, MMR, and RCR genes in the embryo. The ATM stress-response pathway regulates cell cycle arrest after DNA damage. While in yolk sac ATM expression was constant from GD10-12, in the embryo ATM transcripts increased dramatically (~500% on GD12). 4-OOHCPA decreased ATM expression in the embryo but increased it in yolk sac. Thus, DNA repair and stress response gene expression in the conceptus is regulated in a pathway-, developmental stage- and tissue-specific manner. Understanding how the embryo responds to genotoxic stress will assist in the design of strategies to protect the conceptus from insult. (Support: CIHR & FCAR)

1039 APOPTOTIC SIGNALING PATHWAYS IN TERATOGENESIS.

P. E. Mirkes. *Dept. of Pediatrics, Univ. of Washington, Seattle, WA.*

Between 2 and 3% of all live-born infants have a major developmental defect identified at birth. Although the etiology of these birth defects is often unknown, environmental exposures are known to play a significant role. One of the early events in the pathogenesis leading to birth defects, induced by a wide variety of environmental agents, is cell death. The first part of my talk will describe our research showing that teratogens such as hyperthermia, cyclophosphamide, sodium arsenite, and staurosporine all induce an early episode of cell death in exposed day 9 mouse embryos. This cell death is apoptotic in nature and is characterized by the release of mitochondrial cytochrome c followed by activation of a caspase cascade. Thus, these 4 teratogens induce cell death by activating the intrinsic, mitochondrial apoptotic pathway. Teratogen-induced cell death is also selective, i.e., some cells in the embryo die while the majority of cells do not. For example, we have shown that cells of the developing CNS are particularly sensitive to teratogen-induced cell death whereas cells of the developing heart are completely resistant. The second part of my talk will present more recent data showing that this heart cell resistance is associated with a block in the mitochondrial apoptotic pathway. The final part of my talk will present a number of signaling pathways that have been shown to interact with the apoptotic pathway, such as the mitogen-activated protein kinase pathways, the NF-κB pathway, and the PI3-AKT pathway. (Research supported by grants ES 07026 and ES 08744 from the National Institutes of Environmental Health Sciences.)

1040 OLFACTORY TRANSPORT OF INHALED METALS: AN IMPORTANT ROUTE OF DELIVERY TO THE BRAIN.

D. C. Dorman[1] and J. R. Harkema[2]. *[1]CIIT Centers for Health Research, Research Triangle Park, NC and [2]Department of Food Safety and Toxicology, Michigan State University, East Lansing, MI.*

The olfactory system is unique in that it forms a direct interface between the air and the central nervous system (CNS). There is growing evidence that metals deposited within the nose can be absorbed at this site and then undergo transport along the olfactory nerve. The transport of metals within the olfactory nerve can result in direct delivery of inhaled metals to the brain. In some cases, direct olfactory transport is the dominant pathway by which an inhaled metal reaches the CNS. This symposium will discuss what is known about the neuronal transport of metals in the olfactory pathway and will include a discussion of the likely mechanisms by which transport occurs. This symposium will also cover clinical features of metal-induced nasal toxicity in humans and will discuss inter-species differences in nasal anatomy that may play a role in metal olfactory uptake and transport. One metal of special concern to be discussed in this symposium is manganese, a neurotoxic metal shown to be able to cross synapses in the olfactory bulb and migrate *via* secondary olfactory neurons to distant nuclei of the brain.

1041 COMPARATIVE NASAL STRUCTURE, FUNCTION AND TOXICOLOGY: RELEVANCE TO OLFACTORY TRANSPORT OF METALS.

J. R. Harkema. *Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI.*

In laboratory animals, several metals (e.g., Al, Cd, Mn, Ni, Zn) pass *via* olfactory neurons from the nasal airway lumen to distant sites in the brain. Exposure to metal-containing compounds (e.g., $NiSO_4$, $ZnSO_4$) may also cause marked toxicity of the olfactory mucosa. Differences in the nature and magnitude of metal-induced responses (physiologic or pathologic) of the olfactory mucosa to inhaled metals among mammalian species may be due to species-specific differences in nasal airway structure and function. The purpose of this introductory lecture is to provide a concise review of the similarities and differences in nasal anatomy, physiology, and toxicologic pathology among laboratory animals and humans, emphasizing important comparative aspects of the olfactory mucosa. Rodents are obligate-nose breathers and approximately half of their nasal lumens are lined by olfactory neuroepithelium with direct neuronal connections to the olfactory bulb. In contrast, primates are oronasal breathers with only a small amount of their nasal lumens lined by olfactory epithelium. These species differences along with differences in nasal airflow may translate into differences in intranasal deposition of inhaled metals and subsequent metal transport to the brain and/or olfactory toxicity. In addition, injury, adaptation and repair of the olfactory epithelium may markedly alter metal transport to the brain. Knowledge of comparative nasal structure, function and toxicity is essential for interpreting animal data and estimating the potential risks of nasal or neurological damage in humans exposed to inhaled metals.

1042 METAL TRANSPORT IN THE OLFACTORY SYSTEM.

H. Tjälve. *Pharmacol. & Toxicol., Swedish University of Agricultural Sci., Uppsala, Sweden.* Sponsor: D. Dorman.

We have examined the transport of several metals (manganese, cadmium, nickel, mercury, zinc and cobalt) in the olfactory system of rats and pike. Among these metals manganese has been found to have a unique capacity to be taken up *via* the olfactory pathway. Thus, following transport along the primary olfactory neurons to the olfactory bulbs, manganese continues *via* other neurons to all parts of the brain and even into the spinal cord. Studies with nickel, zinc and cobalt indicate that these metals slowly pass from primary to secondary olfactory neurons. Cadmium and mercury are transported along the primary olfactory neurons but appear unable to continue along secondary olfactory neurons. Studies in pike indicate that cadmium and manganese move along the primary olfactory neurons by fast axonal transport, whereas nickel moves by slow axonal transport. We assume that the metals will adhere to proteins or some other endogeneous neuronal con-

stituent(s) undergoing neuronal transport. Many metals have a strong affinity for SH-groups of proteins, which may render them prone not to leave the primary olfactory neurons, or to do so very slowly. Recent studies indicate that cadmium in rats and pike is transported along the olfactory neurons bound to metallothionein. Unlike many other metals manganese does not possess a high affinity for any endogeneous ligand, such as e.g. SH-groups, and may therefore move relatively easily between different tissue constituents within the olfactory system, as well as in the brain. We consider that the neurotoxicity seen at occupational inhalation of manganese-containing dusts or fumes may be due to an uptake of the metal into the brain *via* the olfactory pathway. We have recently shown that manganese taken up *via* the olfactory pathway in rats will affect astrocytes, indicating that these cells may be the primary targets of manganese in the CNS and that the adverse effects of the metal on neurons are secondary to impaired function of the astrocytes.

1043 OLFACTORY TRANSPORT OF INHALED MANGANESE.

D. C. Dorman. *CIIT Centers for Health Research, Research Triangle Park, NC.*

Research efforts examining the dosimetry of inhaled manganese primarily focus on pulmonary deposition and subsequent hematogenous delivery of the metal to the central nervous system. Growing evidence suggests that nasal deposition and transport along the olfactory nerve represents a direct route by which manganese is delivered to the olfactory bulb and the telencephalon *via* transport in secondary olfactory neurons. Until recently, little was known regarding olfactory transport of manganese following inhalation exposure. We have conducted studies in rats using short-term (90-min) inhalation exposure to radiolabeled manganese aerosols ($54^{Mn}{}_{Cl2}$ or $54^{Mn}HP_{O4}$). These studies used an animal model in which one nostril was occluded thus preventing olfactory transport of manganese to one side of the rat brain. We found that the olfactory route contributes the majority (> 90%) of the 54^{Mn} found in the olfactory pathway of the brain following acute inhalation exposure. However, neither of these inhalation studies clearly demonstrated that olfactory uptake contributes significantly to increased striatal manganese concentrations. Results of 14-day inhalation studies in rats exposed to relatively soluble or insoluble manganese aerosols further demonstrate that manganese concentrations achieved in the olfactory bulb are significantly higher than that observed in either the striatum or cerebellum, lending credence to the direct olfactory transport theory. The relevance of these findings to human manganese inhalation exposure and the risks for neurotoxicity are not known and are complicated by interspecies differences in nasal and brain anatomy and physiology. In the rat, the olfactory bulb accounts for a relatively large portion of the central nervous system, and the nasal olfactory mucosa covers approximately 50% of the total nasal epithelium. These structures are proportionately smaller in humans, suggesting that this route of brain delivery may be less important in humans as compared to the rat. These differences likely predispose the rat, more so than humans, to olfactory deposition and potential olfactory transport of manganese.

1044 NASAL TOXICITY OF METALS IN HUMANS.

F. W. Sunderman, Jr[1]. [1]*Middlebury College, Middlebury, VT and* [2]*University of Vermont, Burlington, VT.*

Occupational exposures of workers to inhalation of certain metal dusts or aerosols can cause diverse forms of nasal toxicity, including loss of olfactory acuity, atrophy of the nasal mucosa, perforated nasal septum, or sinonasal cancer. Anosmia and hyposmia have been reported in workers exposed to Ni- or Cd-containing dusts in alkaline battery factories, nickel refineries, and cadmium industries. Anosmia has also developed in children following experimental nasal spraying with zinc sulfate solution. Ulcers of the nasal mucosa and perforated nasal septum have been reported in workers exposed to Cr(VI) in chromate production and chrome plating, or to As(III) in arsenic smelters. Cancers of the nose and nasal sinuses have been reported in workers exposed to Ni compounds in nickel refineries, cutlery factories, and alkaline battery manufacture, or to Cr(VI) in chromate production and chrome plating. In humans, the olfactory bulb contains an abundance of certain metals (Cd, Mn, Hg) compared to other regions of the brain, which is consistent with experimental evidence in animals that metals can enter the brain *via* the olfactory tract.

1045 INTRODUCTION: THE UNFILLED PROMISE OF BIOMARKERS.

H. Zenick. *National Health and Environmental Effects Research Laboratory, Office of Research and Development, USEPA, Research Triangle Park, NC.*

The 1983 NRC publication formalized human health risk assessment into a four-component process, namely, exposure assessment, hazard identification, dose-response assessment, and risk characterization. Since that report, research has focused on addressing scientific uncertainties within each of these components. Less emphasis has been given to research to strengthen the linkages between these elements to better understand the health risks associated with actual human exposures. The emergence and pursuit of biomarkers offered the promise of being able to measure signals and/or events that reflected more accurately the biology associated with exposure, effects, and susceptibility. However, much of this work remains disconnected rather than integrated. The cost of failing to conduct more integrative research can be illustrated by considering the recent CDC Human Exposure Report. In that report, blood and urine levels for 27 chemicals are presented; yet, save for lead and cotinine, the ability to interpret these values is quite limited (e.g., identify significant pathways and/or potential health risks). However, the report does provide a timely opportunity to reexamine the scientific issues and opportunities accompanying what will become an expanding, annual data base. This session will serve as a catalyst for such dialogue through a series of presentations to define the research needed to validate and translate such biomarker data. Following a review of the current and future scope and design of the CDC program, a case history will be presented that uses blood lead to demonstrate the attributes of a successful biomarker. The potential role of pharmacokinetic modeling to link exposure and effects biomarkers will be considered followed by an examination of the challenges to toxicology and epidemiology to provide the research and insights vital to interpreting biomarker data similar to that being generated by CDC. This abstract has been subjected to Agency for review and approval for publication.

1046 CDC'S BIOMONITORING PROGRAM: THE NATIONAL REPORT ON HUMAN EXPOSURE TO ENVIRONMENTAL CHEMICALS.

E. J. Sampson. *Division of Laboratory Sciences, Centers for Disease Control and Prevention, Atlanta, GA.* Sponsor: H. Zenick.

The Centers for Disease Control and Prevention (CDC) has established a National Report on Human Exposure to Environmental Chemicals that provides biomonitoring data from participants in the National Health and Nutrition Examination Survey (NHANES). In the Report, CDC measured 27 chemicals in blood and urine samples from this ongoing national survey of the United States population. The chemicals included metals (e.g., lead, cadmium, mercury, uranium), cotinine (a metabolite of nicotine), 6 organophospate pesticide metabolites, and 7 phthalate metabolites. Highlights of the Report show that blood lead levels among children have declined since the early 1990's and that there has been a major reduction in exposure of the US population to environmental tobacco smoke. In addition, the Report provides new data on children's and women's exposure to mercury and first time data on exposure in the general population to 24 chemicals. The Report will be updated each year with new data for the general population. For example, next year CDC will combine the 1999 survey data with new data from participants in NHANES 2000 to provide updated national estimates on the 27 chemicals. Plans are to expand the report with additional chemicals each year, eventually exceeding 100 chemicals measured on the population per year. Chemicals under consideration for future Reports include volatile organic compounds, carcinogenic polyaromatic hydrocarbons, dioxins, furans, polychlorinated biphenyls, carbamate pesticides, and organochlorine pesticides. Future additions to the Report will provide more detailed assessments of exposure levels among different population groups defined by sex, race/ethnicity, age, urban/rural residence, education, income and other characteristics.

1047 BLOOD LEAD LEVELS AS BIOMARKERS AND SURVEILLANCE INDICATORS.

E. K. Silbergeld. *Program on Human Health and the Environment, University of Maryland, Baltimore, MD.*

The National Health and Nutrition Examination Survey (NHANES) is a periodically conducted nationally representative surveillance of the US population. Its data on national blood lead levels in the US population have been among the most successful biomarkers for environmental health ever undertaken. In NHANES II (conducted from 1976 to 1980) the first national prevalence data on lead exposures revealed the extent of excess lead exposures and the socio-demographic determinants of environmental racism related to lead. These data were influential in the campaign to remove lead from gasoline, culminating with the 1990 amendments to the Clean Air Act. These data have also stimulated attempts at reducing sources of lead in housing and urban environments. The NHANES III (1991-1994) demonstrated the effectiveness of these policy interventions by showing significant reductions in blood lead levels among all subgroups of the US population (age, gender, ethnicity, income, and residence), but these data also showed the continued disparities in lead risks for US children under 6 years. NHANES data have also been used to generate important hypotheses on lead toxicity. Using NHANES data, studies have associated lead with decreased growth in stature, deficits in hearing, and increased blood

pressure. These data have also suggested a potential role for menopause in increasing blood lead. These blood lead data are powerful for two reasons: (1) the surveillance program is carefully designed for national relevance, and (2) toxicology allows us to draw inferences between the biomarker and health risks. Thus, well understood biomarkers collected in well designed studies can be extraordinarily useful in both research and public policy.

1048 BIOMARKERS AND PHARMACOKINETIC MODELING: THE KEY TO ESTABLISHING THE EXPOSURE-DOSE-EFFECTS LINKAGE.

M. L. Rigas[1], H. A. Barton[2] and M. S. Okino[1]. [1]*National Exposure Research Laboratory, USEPA, Las Vegas, NV and* [2]*NHEERL, USEPA, Research Triangle Park, NC.*

Measures of chemical in tissues, blood, or urine are useful to the extent that they can be related to health effects and because they are an integrated measure of absorbed dose following exposure. However, the direct relationship between biomonitoring data and pathway-specific exposures is more tenuous. Pharmacokinetic (PK)models are mathematical representations of, at minimum, absorption and clearance and can estimate internal dose based on multi-route exposure. The reverse calculation to extract a unique exposure profile based on biomonitoring data is not possible. PK models can provide useful information relating biomonitoring data to exposure using additional data such as those generated by human exposure field studies to aid in these estimates. There are several methods that use PK models and biomarker data to further understand the timing and magnitude of exposures. In one case, we have explored this relationship using the urinary metabolite of the organophosphate insecticide chlorpyrifos collected as part of a pesticide exposure study in children. To set bounds for the modeling, we assumed that the children received some constant low-level background exposure and were subjected to intermittent higher level exposure events as consequence of pesticide application in the home. Using a PK model and a mathematical algorithm to constrain possible exposures based on survey data of pesticide usage by the families, we calculated plausible exposure scenarios that would lead to the urinary biomonitoring results. It is also important to consider how the pharmacokinetic properties (e.g. elimination half life) of a compound are related to the certainty bounds of the exposure estimation from biomarker data. (The USEPA, through its Office of Research and Development, funded this research. The abstract has been subjected to Agency review and approved for publication.)

1049 IMPLICATIONS OF BIOMARKER DATA FOR THE FUTURE OF TOXICOLOGY AND RISK ASSESSMENT.

J. S. Bus. *Toxicology & Environmental Research & Consulting, The Dow Chemical Company, Midland, MI.*

The CDC's Annual Human Exposure Report will provide valuable exposure information impacting the future design and interpretation of toxicology studies, and also assure continued improvements in the integration of this toxicology and human exposure data into risk assessments. Data describing human body burdens of chemicals will be provide pivotal inputs to future dialog surrounding potential expansion and/or alteration of conventional toxicology testing protocols to more reasonably mirror real-world exposures (e.g., implications for use of traditionally-defined MTD's in toxicity hazard testing; design of mixture studies; etc.). However, the Annual Report would be of even greater value to the debate if it were expanded to include analyses of natural products. Such comparative perspectives (synthetic *vs.* natural) could significantly inform future directions of risk assessment, e.g., what represents appropriate Uncertainty Factors in Margin-of-Exposure based risk assessments. The Annual Report also will provide data critical for effective validation and refinement of human exposure models, improving their ability not only to more accurately estimate potential exposures resulting from manufacturing, distribution, and use of chemicals, but also to more effectively measure the efficacy of regulatory and/or product stewardship decisions. Despite the enormous potential of the Annual Report data to augment the future of toxicology and risk assessment, there must be concurrent emphasis that such biomonitoring data does not by itself indicate risk, i.e., it is the dose, not just the presence, that makes the poison.

1050 DRUG-INDUCED VASCULAR DISEASE: MARKERS OF INJURY.

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Injury to vascular structures is a relatively common hazard identified in preclinical drug toxicity studies. The mechanism of action of novel compounds which cause vascular injury do not always fit accepted dogma that relates cardiovascular damage to marked hypotension and reflex tachycardia. Terminology, relative to classification of vascular lesions by pathologist and clinicians, also presents communication issues since the term vasculitis evokes differing images, mechanisms and consequences to scientists of differing backgrounds. Historically, studies in animals have not predicted the drug-induced vasculopathies observed in humans, nor have the drug-induced vascular lesions recognized in animals been associated with clinical consequences. Collectively, these issues culminate in confusion when attempting to predict potential hazards of new drugs in a clinical setting and emphasize the need for non-invasive markers that can be used preclinically and clinically to monitor for development of drug-induced vascular injury. The lack of markers for vascular injury has caught the attention of toxicologists, pathologists, vascular biologists, regulators and clinicians. This dilemma has prompted significant investigation, with novel approaches, to identify circulating markers predictive of damage to endothelial cells or cells of the vascular wall. Investigations have employed a spectrum of antibody-based, flow cytometric assessments or magnetic resonance spectroscopy-base technologies on various body fluids and tissues as well as traditional plasma-based evaluation of endothelial products. The purpose of this workshop will be to briefly discuss the possible approaches for detection of vascular injury, the terminology to facilitate common understanding and communications and to have in depth discussions of new markers, technical approaches and their promise for future application.

1051 PLASMA MARKERS OF VASCULAR INJURY: NEW TOOLS FOR THE TOXICOLOGIST AND CLINICAL PHARMACOLOGIST.

A. Blann. *City Hospital NHS Trust, Birmingham, United Kingdom.* Sponsor: W. Kerns.

Damage to the endothelium is thought to be important in the initial clinical stages of atherosclerosis and in the onset of several connective tissue diseases. Consequently, we have had the opportunity, in a clinical setting, to correlate changes in certain plasma markers with vascular disease and its progression. Among the plasma markers evaluated, reliable, sensitive, and specific markers of endothelial function in humans include von Willebrand factor, soluble E selectin and soluble thrombomodulin. Each represent different aspects of the physiology and cell biology of the vessel wall, the pathophysiology of the disease process and development of clinical events. Measurement of these markers by ELISA has helped provide essential contributions to our understanding of the development of cardiovascular disease as well as in risk factors (diabetes, hypertension, smoking) and in cancers and the diseases related to rheumatoid arthritis such as systemic sclerosis and systemic lupus erythematosus. For example, increased concentrations of some of these markers predict a poor prognosis such as myocardial infarction and stroke. Notably, von Willebrand factor can also be measured easily in rats, but its value as a possible marker for vascular damage is unknown. Therefore, as the markers have predictive power in helping identify those persons at risk for development or progression of disease, it seems possible that they may prove useful in studying vascular injury in cases of acute (aspirin) and chronic (heavy metals) poisoning. Following a brief review of endothelial cell biology, the predictive nature of various markers will be established from case studies in humans.

1052 USE OF METABONOMIC TECHNOLOGY TO NON-INVASIVELY ASSESS DRUG-INDUCED VASCULAR INJURY.

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As a class, the vasculitides encompass a variety of preclinical and clinical conditions and are, in general, empirically defined and poorly understood because of the lack of specificity of associated signs and symptoms, the inaccessibility of target tissues for pathologic examination, and importantly, the absence of efficient non-invasive diagnostic tests. Metabonomics is a recently emerging approach that combines high-resolution nuclear magnetic resonance spectroscopy (NMR) of biofluids with pattern recognition technology to evaluate the metabolic status of an organism. The underlying principle of metabonomics, as with traditional biomarkers of disease, is that homeostatic alterations during disease processes or toxic insult may be reflected directly or indirectly in body fluids, and may ultimately leave biomolecular traces in urine. The advantage of metabonomics lies in its capacity to monitor simultaneously and non-invasively tremendous numbers of trace molecules. Metabonomic evaluation of urine collected from animals given model compounds has demonstrated the utility of this technology to detect changes induced by drugs that cause vascular injury. Appropriate discretion is advised as the technique is sensitive to unrelated pharmacologic and clinical effects that can complicate interpretation of the NMR spectra and the challenge remains to link specific changes with specific types of vascular injury. Nonetheless, the technology has shown significant promise for sensitively and selectively identifying animals with vascular injury by analysis of

urine samples. Apparent thresholds were tentatively identified and the ability to evaluate the complete temporal response has provided unique mechanistic insights into this troublesome lesion.

1053 PHOSPHODIESTERASE TYPE III INHIBITOR-INDUCED VASCULAR INJURY IN RATS: POTENTIAL BIOMARKERS OF VASCULAR DAMAGE.

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A number of pharmacologic agents including dopaminergic agonists, reverse transcriptase inhibitors, phosphodiesterase inhibitors (PDEI's), and endothelin receptor antagonists induce, in a number of animal species, histopathologic evidence of vascular injury, a toxicity that has vexed both pharmaceutical developers and regulators. Consensus approaches are needed to establish the relevance or lack of relevance of such animal findings to humans. Uncertainty in drug development and regulatory decision-making could be reduced if specific signals heralding the earliest onset of events leading to vascular injury could be identified and monitored appropriately. Biomarkers that identify the onset of drug-induced vascular injury have not been established. We have treated several strains of rats with SK&F 95654, a PDEI III, and observed a spectrum of lesions in the mesenteric vascular bed with SHR being highly responsive and WKY fairly resistant. We have applied 2-d gel electrophoresis and SELDI to sera protein samples; screening antibodies, flow cytometry, and microarrays to circulating peripheral blood leukocyte samples; as well as metabonomics to urine samples from these studies, with the goal of identifying some parameter(s) that could serve as accessible biomarker(s). The data indicate that certain changes are consistently associated with drug-induced vascular injury. Defining the full practical utility of such potential biomarkers will require a careful assessment of the strength of their association with alterations in histopathology, including an assessment of drug sensitivity and specificity limitations using additional classes of agents across species.

1054 MECHANISTIC STUDIES OF PHOSPHODIESTERASE INHIBITOR INDUCED VASCULAR DAMAGE AND UTILITY OF CIRCULATING ENDOTHELIAL CELLS AS MARKERS OF VASCULAR INJURY.

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Studies of drug-induced vascular injury in animals have emphasized species differences, and vascular bed sensitivities to injury. Further, there are no widely accepted markers predictive of vascular injury in animals. Phosphodiesterase inhibitors are known to induce vascular injury in rats providing a useful model, but vascular injury is generally only recognized after necropsy and/or histologic examination. Differences in species susceptibility to drug-induced vascular damage has been correlated to differences in species pharmacologic responsiveness *in vitro*. Phosphodiesterase 4 (PDE4) inhibitors induce segmental mesenteric arterial lesions when administered to rats, but not rabbits or monkeys, even though exposure in rabbit and monkey were substantially higher than exposures associated with vascular injury in the rat. Vasorelaxant properties of a PDE4 inhibitor were compared in mesenteric arterial ring preparations from rats, monkeys and rabbits. The EC_{50} for relaxation was 18.0 μM in rats and > 100 μM in monkeys, and did not induce relaxation in rabbits. In addition, regional differences in the distribution of PDE4 isozymes have been explored to explain segmental distribution of mesenteric arterial lesions in rats. The PDE4 isozymes evaluated by confocal laser scanning microscopy were PDE4 A, B, C and D. Distribution differences in various isozymes apparently do not correlate with the segmental distribution of vascular injury. Quantitation of circulating endothelial cells may be a useful biomarker of vascular injury since numbers are known to increase after various physical, infectious and chemical insults to the vasculature. The usefulness of circulating endothelial cells as a potential biomarker for vascular injury in the rat has been evaluated using flow cytometric methodology. Results with selected drug-induced vascular insults will be summarized. In summary, continued investigation holds promise for further defining mechanisms of species and vascular bed sensitivities and to provide markers of vascular injury.

1055 EXAMPLES OF RODENT NONGENOTOXIC MECHANISMS OF NEOPLASIA: ARE THESE RELEVANT TO HUMAN RISK ASSESSMENT?

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Numerous compounds (*e.g.*, agricultural products, commodity chemicals, and pharmaceutical agents) have been shown to induce neoplasia *via* nongenotoxic mechanisms when assessed for safety in two-year mouse and/or rat carcinogenicity studies. In many instances, the neoplasia has been attributed to disruption of normal physiology. When this disruption is sustained, a series of well-characterized changes occur in the target organ including hypertrophy, hyperplasia, and eventually neoplasia. The ovary (granulosa cell), pancreas (acinar cell), testis (Leydig cell), thyroid (follicular cell), and stomach (endocrine cell) were selected because sufficient investigative data exist to characterize the mode-of-action for several compounds. In addition, sufficient understanding and experience exist to provide context for their relevance to human risk assessment. Each speaker will describe the physiology of the target organ and provide examples of mode-of-action by which compounds disrupt physiologic function. A primary focus will be discussing the comparative differences of structure and function of these selected target organs between rodents and humans, as well as relating the findings to human endocrine disease states and epidemiology to assess whether these nongenotoxic mechanisms of neoplasia are relevant to human risk assessment.

1056 SECONDARY MECHANISMS IN THYROID CARCINOGENESIS.

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Numerous studies have reported that chronic treatment of rodents with goitrogenic compounds results in the development of tumors derived from thyroid follicular cells. Many goitrogenic xenobiotic chemicals increase the incidence of thyroid tumors in rodents by a direct effect on the thyroid gland to disrupt one of several steps in the biosynthesis, secretion, and metabolism of thyroid hormones. This includes (a) inhibition of the iodine trapping mechanism, (b) blockage of organic binding of iodine and coupling of iodothyronines to form thyroxine (T4) and triiodothyronine (T3), and (c) inhibition of thyroid hormone secretion by an effect on proteolysis of active hormone from the colloid. Another large group of goitrogenic chemicals disrupt thyroid hormone economy by increasing the peripheral metabolism of thyroid hormones through an induction of hepatic microsomal enzymes. This group includes CNS drugs, calcium channel blockers, steroids, retinoids, chlorinated hydrocarbons, polyhalogenated biphenyls, and enzymes inducers. Thyroid hormone economy also can be disrupted by xenobiotics that inhibit the 5'-monodeiodinase that converts T4 in peripheral sites to biologically active T3. Inhibition of this enzyme by iodine-containing compounds such as FD&C Red No. 3 lowers circulating T3 levels, which results in a compensatory increased secretion of thyroid stimulating hormone (TSH), follicular cell hypertrophy and hyperplasia, and an increased incidence of follicular cell tumors in rats. Physiologic perturbations alone, such as the feeding of an iodine-deficient diet, partial thyroidectomy, natural goitrogens in certain foods, and transplantation of TSH-secreting pituitary tumors in rodents also can disrupt thyroid hormone economy and, if sustained, increased the development of thyroid tumors in rats. A consistent finding with all of these goitrogens is the chronic hypersecretion of TSH which places the rodent thyroid gland at greater risk to develop tumors through a secondary (indirect) mechanism of thyroid oncogenesis associated with hormonal imbalances. The human thyroid is much less sensitive to this pathogenetic phenomenon than rodents.

1057 MECHANISMS OF HORMONE-MEDIATED CARCINOGENESIS OF THE OVARY.

C. C. Capen. *Department of Veterinary Biosciences, The Ohio State University, Columbus, OH.* Sponsor: J. Cook.

The objectives of this presentation are to: 1) discuss selected examples of non-genotoxic endocrine mechanisms of ovarian carcinogenesis in rodents, and 2) consider the relevance of rodent ovarian tumor development for human risk assessment. Factors that destroy or greatly diminish the number of ovarian follicles in rodents include senescence, genetic deletion of follicles, X-irradiation, a wide variety of marketed drugs and xenobiotic chemicals, transplantation of ovaries into the spleen, and early thymectomy with the development of autoantibodies to oocytes. All are known to diminish sex steroid hormone secretion by the ovary. Other mechanisms include xenobiotic chemicals that bind to the estrogen receptor in the hypothalamus, (*e.g.* selective estrogen receptor modulators such as Raloxifene), genetic deletion of the estrogen receptor (ER)-α (ER knockout mice), and transgenic mice expressing chimeric luteinizing hormone (LH)-β subunit. The resulting elevated circulating levels of gonadotrophins, especially (LH), due to disruption of negative feedback on the hypothalamus-pituitary axis promotes ovarian tumor development in rodents. For example, the long-term receptor-mediated stimulation of stromal (interstitial) cells by LH and indirectly the ovarian surface epithelium places the mouse ovary at increased risk for developing unique tubular (tubulo-stromal) adenomas. The formation of similar complex ovarian tumors in genetically sterile mice *not* exposed to exogenous chemicals and the prevention of tumor formation by estrogen administration or implantation of normal ovaries supports a secondary (hormonally mediated) mechanism of ovarian oncogenesis. The intense proliferation of ovarian surface epithelium and stromal cells with frequent development of complex

ovarian tumors in rodents as a response to sterility does not have a counterpart in the ovaries of women and is not a useful predictor for the development of ovarian neoplasia.

1058 RODENT LEYDIG CELL TUMORIGENESIS AND THE RELEVANCE TO HUMAN HEALTH.

J. C. Cook. *Global Research & Development, Pfizer, Groton, CT.* Sponsor: J. Cook.

Leydig cells (LCs) are the cells of the testis which have as their primary function the production of testosterone, and are a common target in rodent carcinogenicity bioassays. To date, 7 mechanisms for the chemical induction of Leydig cell tumors (LCTs) have been elucidated. Most of these mechanisms involve, ultimately, elevation in serum luteinizing hormone (LH). In contrast to the rat, LH does not appear to be a primary driver of LCTs in the mouse. In the mouse, estrogen receptor agonists/antagonists are the primary driver of LCTs, due to their direct mitogenic response at the LC. Hence, the induction of LCTs in mice does not appear to be relevant to humans due to the different responses at the hypothalamus, pituitary, and testis. However, pathways for regulation of the hypothalamo-pituitary-testis (HPT) axis of rats and humans are similar, such that compounds which either decrease testosterone or estradiol levels or their recognition will increase LH levels. Although, there are several lines of evidence which suggest that human LCs are quantitatively less sensitive than rats in their proliferative response to LH, and hence, in their sensitivity to chemically-induced LCTs. This evidence includes the following: (1) the human incidence of LCTs is much lower than in rodents; (2) several comparative differences exist between rat and human LCs which may contribute to the greater susceptibility of the rat to both spontaneous and xenobiotic-induced LCTs; (3) endocrine disease states in man underscore the marked comparative differences that exist between rats and man in the responsiveness of their LC's to proliferative stimuli; and, (4) several human epidemiology studies are available on a number of compounds that induce LCTs in rats which demonstrate no association between human exposure. This weight of evidence suggests that nongenotoxic compounds which induce LCTs in rats may have low relevance to humans because humans are quantitatively less sensitive than rats to the mitogenic effects of LH.

1059 NONGENOTOXIC MECHANISMS OF EXOCRINE PANCREATIC CARCINOGENESIS IN RODENTS AND THEIR RELEVANCE TO HUMAN HEALTH.

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Spontaneously arising neoplasms in the exocrine pancreas of the rat are almost exclusively acinar cell tumors. Most genotoxic carcinogens that affect the pancreas induce acinar cell neoplasms that are histologically similar to spontaneous tumors. Several nongenotoxic dietary additives including trypsin inhibitors and peroxisome proliferators, as well as high fat diets, have increased the incidence of acinar cell neoplasms in the pancreas of rats. Most of these also promote the development of neoplasms in animals pretreated with genotoxic pancreatic carcinogens. It seems likely that the nongenotoxic dietary additives are promoting the growth of spontaneously-initiated clones of acinar cells which are manifest as focal hyperplasia in "control" rats. The mechanism of the promoting effect of high fat diets is not known, however promotion by trypsin inhibitors and cholestatic agents has been linked to an increase of cholecystokinin (CCK) secretion. CCK serves as a mediator of enyzyme secretion and as a trophic factor for the pancreas in rats. Injection of exogeneous CCK stimulates pancreatic growth (hyperplasia) and promotes carcinogenesis in carcinogen-treated animals. The feedback loop that stimulates excess CCK has been delineated and linked to intraluminal inhibition of trypsin activity or diminished bile secretion. In humans fewer than 1 per cent of pancreatic carcinomas are of acinar cell type and about 90 per cent have a ductal phenotype. Exocrine secretion is largely under neural control in humans, and it is uncertain that CCK is trophic for human pancreas. Some data suggest that human acinar cells express few receptors for CCK. These species differences raise a question as to the relevance of CCK-mediated promotion mechanisms in the human. Furthermore, there is no epidemiologic evidence linking ingestion of trypsin inhibitors to increased risk of pancreatic carcinoma.

1060 GASTRIC ENTEROCHROMAFFIN CELL-LIKE (ECL) CARCINOIDS IN RODENTS AND THEIR RELEVANCE TO HUMAN DISEASE.

I. Modlin, M. Kidd, K. Lye and T. Hinoue. *Surgery, Yale University School of Medicine, New Haven, CT.* Sponsor: J. Cook.

Clinically, gastric carcinoids develop as a component of atrophic gastritis or as part of the multiple neuroendocrine neoplasia (MEN)-1 syndrome (both types due to hypergastrinemia) or as sporadic lesions. Agents that cause hypergastrinemia and stimulate ECL cell proliferation have been investigated in a number of conventional models (rat, mouse) as well as the Mastomys and Japanese cotton rat. Pharmacological doses (100 x human levels) of H2 receptor antagonists or proton pump inhibitors result in ECL cell hyperplasia (8-10 wks) and eventually neoplasia (>2yrs) in rats, mice and cotton rats. A number of agents commonly found in the human diet (methyl eugenol), used as anti-lipidemic drugs (ciprofibrate) or in crop preservation (butachlor) are all tumorigenic and cause ECL cell carcinoids in rodents; effects due to gastric mucosal damage and hypergastrinemia. In contrast, in the Mastomys, the standard therapeutic doses of an acid suppressive agent result in ECL cell hyperplasia (6-8 wks) with tumor formation by 4 months. Tumor development in this rodent and in man share a number of common traits. Carcinoids may spontaneously develop over the life-time of both species. These tumors are somatostatin receptor type 2 (SSTR2) positive. Somatostatin (octreotide) therapy prior to acid perturbation inhibits tumor formation in this animal, while *in vitro* studies demonstrate that this may affect both ECL cell secretion as well as negatively regulate proliferation. Human and Mastomys tumors also share alterations in growth factors (TGF-alpha), in p53 (overexpressed) and in the menin gene (somatic mutations). One difference is in the ECL cell growth response to acid suppression; a doubling of ECL cell numbers is seen in humans, while neoplasia is most often seen in Mastomys. The underlying genomic alteration (s) is unknown. The substantial overlap in tumor pathophysiology between the Mastomys and the human suggest that this rodent may specifically be an important model for investigating nongenotoxic gastric carcinoid formation.

1061 AH RECEPTOR ACTIVATION REQUIRES INDUCTION OF MAP KINASE PATHWAYS BY TCDD.

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TCDD is a widespread environmental contaminant that causes adverse toxic effects in animals and humans. Toxicity is mediated by activation of the cytosolic aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor essential for TCDD-induced expression of several phase I and phase II drug metabolizing enzymes. TCDD also activates signal transduction pathways, but the molecular mechanisms by which these pathways may contribute to AHR activation and TCDD toxicity have yet to be defined. Here we report that TCDD induces in mouse hepatoma Hepa-1 cells the immediate activation of extracellular signal-regulated kinases (ERKs) and Jun N-terminal kinases (JNKs), two subgroups of the mitogen-activated protein kinase (MAPK) family involved in modulation of transcription factor activity and regulation of gene expression. Our results show that MAPK activation by TCDD is not mediated by the AHR, since it takes place in CV-1 cells and in *Ahr(-/-)* mouse embryonic fibroblasts, both of which lack AHR expression. Unlike treatment with serum factors or tumor promoters, TCDD-stimulated MAPK activities did not result in the transcriptional activation of ELK and c-JUN but was critical for the induction of AHR dependent gene transcription. Chemical compounds that inhibit ERK and JNK/p38 pathways and dominant-negative effectors of the ERK pathway suppressed induction by TCDD of AHR-dependent reporter gene expression. In addition, TCDD-induced CYP1A1 expression was also partially suppressed by inhibitors of the ERK or the JNK pathways. Inhibition of CYP1A1 induction was maximal when both pathways were blocked, suggesting that the ERK and the JNK pathway each make unique contributions to AHR activation. These results indicate that induction by AHR ligands of MAPK pathways is an important element in AHR activation of gene expression. *(Supported by NIH 2R01-ES6273 and 1R01-ES10807).*

1062 BRG-1 IS REQUIRED FOR CYTOCHROME P4501A1 TRANSCRIPTION MEDIATED BY THE ARYL HYDROCARBON RECEPTOR COMPLEX.

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Chromatin remodeling is a key step in overcoming the nucleosomal repression of active transcription in eukaryotes. The mammalian SWI/SNF ATP-dependent chromatin-remodeling complexes contain multiple subunits. The major ATPase activities in these complexes are attributable to either BRG-1 or the related brahma(Brm) protein. The aryl hydrocarbon receptor (AHR), after binding xenobiotic ligands such as 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), associates with the AHR nuclear translocator(ARNT), and the dimer activates transcription of several genes, including cytochrome P4501A1(CYP1A1). We show that introduction of BRG-1 into the BRG-1 and hBrm deficient SW13 and C33A human cell lines enhances expression from a transiently transfected AHR/ARNT- dependent reporter gene. We also demonstrate that replenishment of BRG-1 to SW13 cells restores endogenous cytochrome P4501A1 (CYP1A1) expression, whereas an ATPase deficient mutant of BRG-1 is unable to do so. Using CHIP (Chromatin Immunoprecipation), we demonstrate that BRG-1 associates with the enhancer region of the mouse CYP1A1 gene *in vivo* in a TCDD- and ARNT-dependent fashion, suggesting the specific recruitment of BRG1 by AHR/ARNT. Finally we demonstrate that the transcriptional activation domain of AHR interacts with

BRG-1. Together our studies reveal an essential involvement of BRG-1 in activating CYP1A1 gene transcription and implicate the importance of ATP-dependent chromatin remodeling activity in inducible gene expression mediated by AHR/ARNT.

1063 IDENTIFICATION OF HSP90 CHAPERONE COMPONENTS THAT REGULATE ARYL HYDROCARBON (DIOXIN) RECEPTOR SIGNALING.

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The chaperones are a family of highly conserved proteins that play numerous roles in cells. Among these roles, Hsp90 proteins and their co-factors are proposed to regulate the conformation and function of nuclear hormone receptors. Aryl hydrocarbon receptor is a transcription factor that is activated by xenobiotic ligands such as TCDD and requires interactions with Hsp90 proteins for proper function. We constructed a yeast model system that used transcriptional activation by the human aryl hydrocarbon receptor-Arnt protein complex as an endpoint to genetically assess which chaperones and co-chaperones regulate this signaling pathway. Most Hsp90 chaperones and co-chaperones are structurally and functionally conserved between yeast and humans, thus making this a relevant model system for these studies. Genetic evidence from strains lacking particular chaperone and co-chaperone genes indicates that the proteins Hsc82 (a homolog of human Hsp90), Sba1 (a homolog of human p23), and the tetratricopeptide repeat (TPR)-containing proteins Cpr7 and Cns1 have roles in the aryl hydrocarbon receptor signaling pathway. The absence of other Hsp90 and co-chaperone proteins (Hsp82, Cpr6, Ppt1) had no affect in this assay. Thus, specific chaperone and co-chaperone proteins participate in the aryl hydrocarbon receptor signaling pathway. We propose that the cellular levels and interactions of chaperones and co-chaperones may determine the response to TCDD and other compounds that act through the aryl hydrocarbon receptor. Our results suggest that interference with chaperone-aryl hydrocarbon receptor interactions might be used to mitigate the toxicity of TCDD and other compounds that act through the aryl hydrocarbon receptor pathway.

1064 IDENTIFICATION OF DOMAINS REQUIRED FOR ARYL HYDROCARBON RECEPTOR INTERACTION WITH THE RETINOBLASTOMA PROTEIN AND FOR REPRESSION OF E2F-DEPENDENT TRANSCRIPTION.

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TCDD is a tumor promoter and carcinogen in rodents, and epidemiological evidence suggests that it causes certain cancers and cardiovascular disease in humans. Most effects of TCDD result from activation of the cytosolic aryl hydrocarbon receptor (AHR) to a transactivator of Phase I and II drug-metabolizing enzymes. AHR also binds the retinoblastoma protein (RB) and potentiates the RB-mediated active repression of E2F functions required for cell cycle progression. We used pull-down assays to identify AHR domains necessary for interaction with RB. Using AHR deletion mutants, we found that maximal binding of AHR to RB requires two AHR domains, the LXCXE cyclin D1-like motif in the B repeat, and a 100-amino acid region in the glutamine-rich transactivation domain. Pull-down assays with RB deletion mutants showed that full length AHR interacts with the large A/B pocket of RB, the region of interaction for all known LXCXE-binding proteins. In gel shift assays, truncated AHR proteins containing either domain displaced RB from an E2F-RB DNA-binding complex and shifted the mobility of E2F-DP1 complexes, suggesting that AHR may establish interactions with E2F that are independent of RB. We used AHR truncation mutants in transient transfection assays to determine the role of the various AHR domains in AHR-mediated repression of E2F-dependent transcription. These experiments confirmed that the LHCAE domain of AHR is required for this effect and showed that mutants lacking AHR transcriptional activity could still repress RB functions. Transfections in ARNT-negative C4 cells suggest that ARNT is not required for AHR-RB interaction *in vitro*, but is necessary for maximal repression of E2F-dependent transcription. These results suggest that AHR-dependent transcription is not involved in repression of E2F-mediated gene expression and that the AHR may exert a direct effect on E2F function independently of the formation of complexes with RB. *(Supported by NIH 2R01-ES06273)*

1065 ERK ACTIVATION BY GROWTH FACTORS OR EXPRESSION VECTORS RESTORES TCDD-SENSITIVITY IN UNRENEWED-SERUM ADIPOCYTE DIFFERENTIATION OF C3H10T1/2 CELLS.

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C3H10T1/2 mouse embryo fibroblast cells differentiate into adipocytes when treated with insulin, dexamethasone, methylisobutylxanthine and the PPARγ ligand BRL49653 (IDM/BRL). In the presence of fresh fetal bovine serum (FBS), PPARγ expression and adipocyte differentiation are inhibited by 2, 3, 7, 8-tetrachloro-*p*-dioxin (TCDD). However, with unrenewed FBS adipocyte differentiation is TCDD-insensitive. Addition of Epidermal Growth Factor (EGF) had no affect on PPARγ expression or adipocyte differentiation in the absence of TCDD. However, in the presence of TCDD, EGF restored inhibition of adipocyte differentiation in a dose-dependent manner. On the other hand, Fibroblastic Growth Factor and Platelet Derived Growth Factor partially inhibited PPARγ expression and adipocyte differentiation in the absence of TCDD, and also restored TCDD-sensitivity in a dose-dependent manner. Addition of specific pharmacological inhibitors of growth factor receptors during serum stimulation showed that no single growth factor within FBS was responsible for TCDD-sensitivity. Pharmacological inhibitors of ERK activation show that the TCDD-mediated inhibition of adipocyte differentiation is dependent on ERK activation in a narrow time window. Addition of a MEK1 dominant positive vector to cells in unrenewed FBS also restored TCDD-sensitivity to the process of adipocyte differentiation. These data establish that factors from an ERK-activated pathway and a TCDD-activated pathway work synergistically to inhibit PPARγ expression and adipocyte differentiation.

1066 DISRUPTION OF NEPHROGENESIS BY BENZO(A)PYRENE, AN ARYL HYDROCARBON RECEPTOR LIGAND, INVOLVES INTERFERENCE WITH ALTERNATIVE SPLICING AND POST-TRANSCRIPTIONAL REGULATION OF THE WILMS' TUMOR SUPPRESSOR-1 GENE.

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The aryl hydrocarbon receptor (Ahr) is a member of the large basic helix-loop-helix and PAS homology domain family of transcription factors. Members of this family are involved in the regulation of neurogenesis and myoblast differentiation. Unregulated activation of Ahr signaling is associated with hydronephrosis, but the mechanisms responsible for this response are unknown. The present studies were conducted to evaluate the influence of benzo(a)pyrene (BaP) on murine nephrogenesis *in vitro*. Embryonic kidneys were dissected from E11.5 mouse embryos and placed in humid culture chambers. Decreases rates of glomerulogenesis and deficits of cellular differentiation were observed in cultures challenged with 3 mM BaP for 4 days. BaP interfered with podocyte differentiation and formation of glomerular basement membranes. Ahr and Wilms' tumor suppressor (WT1) mRNAs were detected by *in situ* hybridization and reverse transcription polymerase chain reaction (RT-PCR) in differentiated, as well as BaP-treated metanephric cultures. Using specific primes for WT1 (±) KTS splice variants it was found that BaP induces the appearance of the (-) KTS splice variant, although total WT1 mRNA levels were unchanged by BaP treatment. Immunolocalization experiments showed a significant decrease in WT1 protein immunoreactivity in cultures challenged with BaP compared to control counterparts. Western analysis confirmed this finding and identified a unique WT1 isoform in metanephric cultures treated with BaP. Comparison of metanephric cultures from Ahr +/+ and Ahr -/- mice showed that Ahr is required for kidney development, as well as BaP-induced deficits in nephrogenesis. Collectively, these results indicate that activation of AhR signaling by BaP disrupts nephrogenesis *in vitro* by modulation of alternative splicing and post-transcriptional regulation of the WT1 gene. (This work was supported in part by NIH grants ES 04917 and ES 09106).

1067 AHR REPRESSOR: A THIRD MEMBER OF THE VERTEBRATE ARYL HYDROCARBON RECEPTOR (AHR) FAMILY.

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The effects of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds occur *via* the AHR, a member of the bHLH-PAS protein superfamily. A single *AHR* gene has been identified in mammals, while many fish species, including the Atlantic killifish (*Fundulus heteroclitus*) possess two distinct *AHR* genes (*AHR1* and a novel form, *AHR2*). A bHLH-PAS protein closely related to AHR was recently identified in mouse and designated AHR repressor (AHRR) (Mimura et al., *Genes Dev* 1999). Mouse AHRR is induced by 3-methylcholanthrene and represses the transcriptional activity of the AHR. The sequence similarity between AHR and AHRR raised the possibility that AHRR is the mammalian ortholog of fish AHR2. To test this, we investigated the presence of an AHRR ortholog in *Fundulus* and identified a cDNA with high sequence identity to the mouse and human AHRRs. *Fundulus* AHRR encodes a 686 residue protein with a predicted

molecular mass of 75.2 kDa. Phylogenetic analysis shows that AHRRs (mammals and fish) form a third clade within the AHR family, along with AHR1 (mammals and fish) and AHR2 (fish only); these three vertebrate AHR-like genes descended from a single invertebrate AHR. Thus, mammalian AHRR is not the ortholog of fish AHR2. We show here that *in vitro*-expressed AHRR proteins from human, mouse, and *Fundulus* all fail to bind [^{3}H]TCDD or [^{3}H]-beta-naphthoflavone. In transient transfection experiments in COS-7 cells using a DRE-luciferase reporter gene, *Fundulus* AHRR had a concentration-dependent inhibitory effect on TCDD-dependent transactivation by both AHR1 and AHR2. *Fundulus* AHRR mRNA is widely expressed and is inducible by TCDD or PCBs. The *Fundulus* AHRR promoter contains three putative dioxin-response elements. Both AHR1 and AHR2 activated transcription of luciferase driven by the AHRR promoter, and AHRR could repress its own promoter. Thus, AHRR is an evolutionarily conserved, TCDD-inducible repressor of AHR1 and AHR2 function. [NIH Superfund ES07381 and NIH ES06272]

1068 GRIP1 ENHANCES AHR SIGNALING IN HEPA1C1C7 CELLS.

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Exposure to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds produces a variety of tissue and species specific toxic and biological effects, most of which are mediated *via* the aryl hydrocarbon receptor (AhR) signaling pathway. The AhR is a ligand-dependent transcription factor that regulates gene expression *via* its binding to the dioxin responsive element (DRE) as a heteromeric complex with the AhR nuclear translocator (ARNT) protein. The events necessary for transcriptional activation by the DNA-bound AhR complex remain to be elucidated. As part of our effort to identify nuclear factor(s) which can modulate the transcriptional activity of the AhR complex, we have examined the ability of the glucocorticoid receptor interacting protein 1 (GRIP1) cofactor to affect AhR/ARNT-dependent signal transduction. GRIP1, like the AhR and ARNT, contains a basic helix-loop-helix/PAS domain located near the N-terminus and two transcriptional activation domains, AD1 and AD2, near the C-terminus. Cotransfection of Hepa1c1c7 cells with a DRE-reporter construct and a GRIP1 expression plasmid results in a 5-8 fold increase in reporter activity over cotransfection with the empty parent vector alone. However, when these cells were cotransfected with a GRIP1 lacking AD1, reporter activity was not enhanced, suggesting a role for AD1 in the augmentation of AhR signaling. Co-immunoprecipitation and GST pull-down techniques revealed strong interactions between GRIP1 and the AhR in at least two regions. The addition of GRIP1 also enhanced AhR:ARNT:DNA complex formation as assessed by gel retardation analysis. These results demonstrate that GRIP1 acts as a coactivator of the nuclear AhR complex. Supported by NIEHS (ES07072, ES05707 and ES04699).

1069 CROSS-TALK BETWEEN THE ARYL HYDROCARBON RECEPTOR AND SIGNAL TRANSDUCTION PATHWAYS: A ROLE FOR NF-κB.

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Activation of gene expression by 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related chemicals is mediated by the aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor. Previously, a role for protein kinase C (PKC) in AhR signal transduction was demonstrated, with PKC inhibition blocking AhR functionality and phorbol-12-myristate-13-acetate (PMA)-dependent PKC activation enhancing AhR-dependent gene expression. In the present study we have extended these analyses and demonstrate that the PMA enhancement of AhR signaling in mouse hepatoma (Hepa1c1c7) cells is dependent upon the AhR DNA-binding site and is transient, occurring within the first eight hours after PMA treatment. While the enhancing effect appears directly related to gene transcription, the inhibition of PKC is associated with decreases in nuclear AhR and gene expression. PMA is also a potent activator of nuclear factor-κB (NF-κB)-dependent reporter gene expression in these cells. Not only do three chemical inhibitors of NF-κB (pyrrolidinedithiocarbamate (PDTC), (E)-capsaicin (CAPS), and caffeic acid phenethyl ester (CAPE)) block PMA-induced expression from an NF-κB reporter gene, but they inhibit both normal and PMA-enhanced AhR-dependent gene expression. Although gel retardation analysis reveals that inhibition of PKC by chelerythrine chloride reduced AhR nuclear translocation, NF-κB inhibition by PDTC had no effect on nuclear AhR levels. These results demonstrate a role for both PKC and NF-κB in the regulation of AhR-dependent gene expression. (Supported by NIEHS grant ES07685)

1070 TNF-α TREATMENT SUPPRESSES CYP1A1 TRANSCRIPTION BY INHIBITING ACETYLATION OF HISTONE H4 AND PHOSPORYLATION OF THE C-TERMINAL DOMAIN OF RNA POLYMERASE II.

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It has been known that TNF-α and lipopolysaccharide (LPS) suppress the gene expression of cytochromes P450 1A1(cyp1a1). In earlier studies, we demonstrated that activation of NF-KB is a critical event leading to the suppression of cyp1a1 gene expression, thus establishing a cross-interaction between the Ah receptor and NF-KB signal transduction pathways. In the present study, we demonstrated that Ah receptor/NF-KB interactions converges at level of transcription involving two steps of transcriptional regulation: (1) chromatin remodeling and (2) transcriptional elongation by RNA polymerase II (RNA Pol II). Specifically, using cyp1a1 transcription in Hepa1c1c7 cells as a model system, we demonstrated that dioxin treatment (10 nM, 2 hrs) causes significant histone H4 acetylation which is associated with chromatin remodeling activity. TNF treatment (5 ng/ml) markedly suppresses this histone acetylation activity, especially around the TATA box region, suggesting that NF-KB activation inhibits the histone H4 acetylation at cyp1a1 promoter. Furthermore, we demonstrated dioxin treatment causes phosphorylation of the C-terminal domain (CTD) of RNA PolI. The phosphorylation of RNA Pol II is a critical step of transcriptional elongation. TNF-α treatment significantly suppresses the dioxin-induced RNA Pol II phosphorylation, especially at serine 2 of YSPTSPS motif of the RNA Pol II CTD. These results established a mechanism for the inflammatory cytokin-induced inhibition of chytochrome P450 1A1, suggesting the involvement of chromatin remodeling and transcriptional elongation in the "cross-talk" between Ah receptor and NF-KB signaling pathways. Supported in part by NIEHS Grant ES09859 and NIEHS Center Grant ES05022.

1071 MECHANISM OF HORMONAL ACTIVATION OF EARLY GROWTH RESPONSE-1 (*egr-1*) IN BREAST CANCER CELLS.

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Egr-1 is induced by 17β-estradiol (E2) in estrogen receptor (ER)-positive MCF-7 breast cancer cells, and analysis of the *egr-1* gene promoter indicates that there are multiple *cis*-elements that could be activated by ER through genomic and non-genomic pathways. The E2-responsive -650 to +12 region of the promoter does not contain an estrogen response element (ERE), and direct binding of ER to the *egr-1* promoter has not been detected. 5'-Deletion analysis of the promoter showed that the -460 to -164 region of the promoter was essential for E2-responsiveness, and this sequence contains 3 serum response elements (SREs) and two Ets motifs. Identification of E2-responsive motifs was further investigated using a series of deletion constructs (pEgr-1f-i) containing the -460 to -283, -383 to -283, and -460 to -383 regions of the *egr-1* gene promoter, respectively. In transient transfection studies in MCF-7 cells, E2 induced reporter gene activity in cells transfected with pEgr-1f (-460 to -283) (7.5-fold), and only weak induction (< 2-fold) was observed in cells transfected with pEgr-1g (-383 to -283). The remaining constructs were not hormone-responsive suggesting that the upstream SRE was the major site of E2-induced transactivation. These results are similar to those recently reported for non-genomic activation of the proximal SRE in the c-*fos* gene by E2 and, in MCF-7 cells transfected with pEgr-1f, hormone-induced activation was blocked by the MAPK inhibitor PD98059. Current studies are focused on characterizing the SRE-binding transcription factors and the pathway for E2-dependent MAPK activation of Egr-1. (Supported by NIH ES09253 and ES09106)

1072 *IN VIVO* STRESS ACTIVATES JAK-STAT IN LIVER BUT NOT BRAIN.

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The Janus kinase-signal transducers and activators of transciption (JAK-STAT) signaling pathways are believed to be crucial signalling pathways in the physiological changes induced by stress. Various stressors (e.g., UV) have been shown to activate the JAK-STAT pathway, *in vitro*, but only a few reports have examined the JAK-STAT pathway using stress models, *in vivo*. Here C57BL6/J female mice were exposed to restraint, vibration or cold stress. To preserve steady-state phosphorylation, the mice were killed by focused microwave irradiation. The activation state of STAT 3, 5 and 6 was assessed from blots of tissue homogenates probed with phospho state-specific antibodies as well as antibodies directed against the context-independent state of the various STATs to assess their total tissue levels. Quantification

was achieved by densitometry of bands generated by enhanced chemiluminescence. No changes in the activation (phosphorylation) state of any of the STATs were evident in brain; however, large increases in phosphoSTAT3 were evident in liver, with restraint and cold providing the largest magnitude changes (~ 300%). STAT3 levels did not change with any of the stressors. Restraint of adrenalectomized mice caused an even larger increase in phosphoSTAT3 (~ 500%). Immunohistochemical examination of STAT3 confirmed that restraint resulted in the nuclear location (i.e. activation) of STAT3, which was substantially greater in adrenalectomized mice. These data suggest that effects of physiological stressors are mediated through a STAT3 pathway that may involve the HPA axis and/or sympathetic nervous system.

1073 COSTS AND BENEFITS OF COMPLIANCE WITH ALTERNATIVE REMEDIATION STANDARDS AT HEXAVALENT CHROMIUM–CONTAMINATED SITES.

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The New Jersey Department of Environmental Protection (NJDEP) requires varying levels of soil remediation at hazardous waste sites depending on the pathway of exposure. For example, sites which contain hexavalent chromium [Cr(VI)] in soil, must comply with either uniform default or site-specific health-based standards for inhalation, dermal, and oral exposures. NJDEP currently relies on an "averaging" approach when evaluating the increased cancer risk from inhalation of suspended soil (i.e., the 95th percentile upper confidence limit of the mean concentration is not allowed to exceed the inhalation standard) and a "bright line" approach when evaluating allergic contact dermatitis (ACD), an acute effect, from dermal contact with Cr(VI) in soil or puddles (i.e., no single soil sample is allowed to exceed the dermal standard). NJDEP also relies on this latter "bright line" approach when evaluating the long-term non-cancer risks associated with incidental soil ingestion, although this is inconsistent with USEPA risk assessment guidelines. The current analysis evaluates whether the extra costs required to comply with NJDEP's approach are justified by the associated benefits. Cost estimates are based on average costs for site remediation and the difference in the volume of soil requiring treatment to meet the oral standard as either a "bright line" or an "average." Benefits are estimated as the expected change in health risk associated with NJDEP's approach, and assumptions about the population affected and value per fatal and nonfatal event averted. The results of the cost-benefit analysis indicate that NJDEP's approach for site remediation yields negative net benefits—i.e., estimated costs are about $100, 000 per site examined as compared to estimated benefits of about $100. Sensitivity analyses, which incorporate a range of possible costs and benefits, also suggest that NJDEP's approach is not cost-beneficial. These findings are important for evaluating the appropriateness and limitations of alternative approaches for complying with soil remediation standards.

1074 INVESTIGATION OF ASBESTOS IN CONSUMER PRODUCTS: CHILDREN'S PLAYSAND.

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The US Consumer Product Safety Commission (CPSC) regulates 15, 000 types of consumer products, including products made with mined materials. Recently, CPSC staff obtained a convenience sample of six playsand products from three companies in five states. Under contract, two independent laboratories analyzed the sand by polarized light microscopy (PLM) and transmission electron microscopy (TEM) for the presence of asbestos fibers. The bulk of each sample was nonfibrous and consisted of quartz, with smaller amounts of rutile, sphene, zircon, horneblende, rock fragments, and opaque grains. Two samples from the same company contained chrysotile and tremolite asbestos (<0.001%). Fibers were small, about 1 µm long and 0.05-0.2 µm in diameter. Small chamber experiments were conducted to determine if asbestos fibers could be released into the air. Air filters were analyzed by TEM using the USEPA AHERA (Asbestos Hazard Emergency Response Act) protocol. Estimated asbestos fiber release from the two playsand samples was 210, 000-2.5 million fibers per minute. Airborne fibers had the same mineralogy and physical characteristics as fibers detected in the bulk samples. Thus, despite the relatively small proportion of asbestos structures in the sand, large numbers of fibers were released into the air during a simulated play activity. Indoor air modeling based on one hour of play in a daycare or classroom setting resulted in projected air concentrations of 0.03 to 0.35 fibers/mL. Although the staff concludes that the very short fibers detected in these samples are not a health concern, we will continue to monitor playsand and other mineral-containing consumer products for the presence of potentially hazardous asbestos fibers. (The opinions expressed by the authors do not necessarily represent the views of the Commission. This abstract is in the public domain and may be freely copied or reprinted.)

1075 DERMAL ABSORPTION OF PCBs IN RHESUS MONKEYS FROM SOIL CONTAMINATED WITH AROCLOR 1260.

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Dermal absorption is routinely evaluated as a potential pathway for absorption of contaminants when conducting human health risk assessments for Superfund sites. For PCB-contaminated soil, the US Environmental Protection Agency uses a dermal absorption factor of 14%, a factor derived from a 1993 study of the dermal absorption of Aroclors 1242 and 1254 from contaminated soil by Rhesus monkeys. In comparison to the 1993 study, the current study varied several parameters that can influence the dermal absorption of lipophilic hydrocarbons, including soil organic content, soil particle size, skin residence time and contaminant "aging" in the soil. The study consisted of four groups of four female Rhesus monkeys exposed to ^{14}C-radiolabeled Aroclor 1260. One group was exposed intravenously to PCBs in propylene glycol (100% absorption) and 3 groups were exposed dermally to PCB-spiked soil. Two dermal exposure groups were exposed for 12 or 24 hours, respectively, to PCBs that had been aged in soil. These groups exhibited percutaneous absorption of 3.43 ± 0.35% and 4.26 ± 0.52%, respectively, of the applied dose. The remaining dermal group was exposed for 24 hours to soil freshly spiked with PCBs and exhibited dermal absorption of 4.07 ± 0.46% of the applied dose. It has been reported that soil organic content is an important factor in modulating the percutaneous absorption of highly lipophilic compounds from soil. The soil used in the current study had an organic content of 5-6%, a value typical for US soil, and a value that contrasts sharply with the 0.9% soil organic content used in the 1993 study upon which the current 14% dermal absorption factor for PCBs is based. Therefore, the current dermal absorption factor used by EPA appears to substantially overestimate the fraction of PCBs available for absorption from soils with typical organics content.

1076 EPA'S INTEGRATED RISK INFORMATION SYSTEM (IRIS).

S. H. Rieth and A. L. Mills. *USEPA, Washington, DC.* Sponsor: D. Singh.

IRIS is an EPA database that contains consensus scientific positions on potential human health effects that may result from chronic exposure to chemical substances. IRIS assessments present summaries of qualitative and quantitative health effects information, including reference doses (RfDs) and reference concentrations (RfCs) for the noncarcinogenic effects of chemicals and cancer slope factors and unit risks. Quantitative assessments in IRIS are used extensively in combination with specific situational exposure assessment information to evaluate potential public health risks. The IRIS database currently contains assessments for over 500 chemical substances. Approximately 80 assessments are underway or will be initiated in FY 2002; these include assessments for chemicals new to IRIS and updates of existing summaries that provide new scientific information and application of more current risk assessment methodologies. The IRIS Program continues to explore ways to improve its assessments, the process by which the assessments are prepared, and the database itself. Two such initiatives are the re-engineering of the IRIS Web site and an IRIS "Needs Assessment." The Web site (www.epa.gov/iris) was redesigned to be a more user-friendly Web-enabled database, with extensive hyperlinks and search interfaces. Users can request a "QuickView" version, which provides essential values with links to full IRIS summary text and supporting documents. The Needs Assessment is intended to define the needs of the public and EPA program offices/regions for new and revised IRIS health assessments. Issues being examined include: what additional chemical substance assessments are needed; which assessments currently on IRIS are in greatest need of scientific update; what additional types of information would be of value (e.g., acute and subchronic values); and to what extent should EPA collaborate with external parties as a means of developing IRIS assessments. Input from this Needs Assessment will be used by EPA in developing the 2002-5 IRIS agenda and resource needs.

1077 EVALUATION OF NON-CARCINOGENIC EFFECTS OF SOLUBLE NICKEL SALTS.

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A risk assessment for chronic toxicity of soluble nickel(reference dose; RfD) *via* oral exposure was prepared by USEPA in 1987 and is available on EPA's Integrated Risk Information System (IRIS). The RfD was based on a two year feeding study in rats given 0, 100, 1000 or 2500 ppm nickel in the diet (estimated as 0, 5, 50 and 125 mg/kg/day). No significant effects were reported at 100 ppm (5 mg Ni/kg/day). At 1000 ppm, body weight was significantly reduced in both sexes; at 1000 pm, females had significantly higher heart-to-body weight ratios and lower liver-to-body weight ratios than controls. The two year survival was poor, particularly in control

rats, both males and females (84% and 92% mortality, respectively), raising some concern about the interpretation of the results of this study. Using NOAEL of 5 mg/kg/day and an uncertainty factor of 300 (10 each for inter- and intra- species variability and 3 for lack of conclusive reproductive toxicity data) the RfD calculated was 0.002 mg/kg/day. A subchronic study conducted by American Biogenics Corporation, also found 5 mg/kg/day to be a NOAEL. Recently Springborn Laboratories completed a two generation reproductive study in which rats received 0, 1, 2.5, 5.0 or 10 mg/kg/day nickel sulfate hexahydrate (0, 0.22, 0.56, 1.12 or 2.22 mg Ni/kg/day) by gavage. Based on the absence of adverse effects on fertility/fecundity, fetal/neonatal survival and on developmental measures at any of the doses tested, the NOAEL identified in this study was 10 mg nickel sulfate hexahydrate/kg/day (2.2 mgNi/kg/day). Increased fetal loss was observed at higher doses in the range-finding study. Using this NOAEL and an uncertainty factor of 100 (10 each for inter- and intra-species variability) the RfD calculated is 0.022 mg/kg/day which is similar to the one currently on IRIS. The opinions expressed are those of the authors and do not reflect US-EPA policy.

1078 RE-EXAMINATION OF THE PROPOSED ARSENIC (AS) DRINKING WATER REGULATION.

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In 2001, the EPA extended the effective date for the 1/22/01 As drinking water rule until 2/22/02. This enabled EPA to conduct an extensive review on the health effects of As and the cost/benefits of the rule. In addition, EPA asked the National Research Council(NRC) to consider health data published since its 1999 report entitled Arsenic in Drinking Water. The Agency also asked the National Drinking Water Advisory Council (NDWAC) to review EPA's cost calculations and the Science Advisory Board (SAB) to suggest methods to improve benefits analysis. The 2001 NRC panel concluded that recent data suggest that the +3 As metabolites might directly react with DNA and that the risks for bladder and lung cancer could be greater than the estimates in the earlier report. The NDWAC group reviewed the costing methodologies and assumptions underlying the system-size cost estimates as well as the aggregated national estimate of system costs of the As Rule. They also evaluated other costing approaches and critiques that may have an impact on the system costs. They were charged to determine whether there is adequate information to evaluate the basis for the alternate approaches or critiques and note where there is not adequate supporting information. For the benefits estimates, the SAB panel asked EPA to consider the latency after initial exposure and the lag between reduction of exposure to As and reduction of risk for bladder and lung cancer. EPA should also quantify the benefits from prevention of some additional diseases, eg. diabetes mellitus, ischemic heart disease, and skin cancer; increase its valuation of non-fatal diseases; and expand its uncertainty analysis. The Agency is weighing the risks of adverse health effects at different As exposures, the costs of treatments to meet the new As standard and the benefits from limiting As exposure in drinking water to concentrations of 3, 5, 10 or 20 µg As/L; EPA will use the balance among these three factors to set the new As Maximum Contaminant Level. [These opinions are the authors' and not necessarily those of the EPA]

1079 TESTING FOR THRESHOLDS AND CALCULATING THRESHOLD DOSES WITH NONLINEAR DOSE-RESPONSE RELATIONSHIPS IN BIOASSAYS FOR CARCINOGENICITY.

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Nonlinear dose-response relationships are often postulated to be thresholded, but definition, statistical criteria, or estimations of threshold doses (TD) are usually lacking. We propose a procedure to estimate TD, based on a linear extrapolation of the line between the response at the lowest-observed effect level LOEL and the no-observed-effect level NOEL to intersect with the dose axis. It is exemplified for bioassays for carcinogenicity with groups of 50 animals. The following data set was used: incidence 0/50 tumor-bearing animals (TBA) at dose 0; insignificant incidences of 1/50 to 5/50 TBA at dose 1; significant responses with 10, 15, or 20/50 TBA at dose 2. The assumption is that the observed sublinearity is a matter of chance only, in that the true incidence at dose 1 is higher than the observed incidence, while the true incidence at dose 2 is lower than the observed. True incidences were calculated by attributing a given percentile of a binomial distribution to the observed incidences. Symmetrical 80% confidence intervals were chosen, which results in a p=0.1*0.1=0.01. Linear extrapolation of the line that connects the true incidences at dose 1 and 2 resulted in the following dose levels at intersection with the dose axis: For 10/50 TBA at dose 2, incidence 1/50 at dose 1 resulted in an intersection at 0.11 dose units; for higher incidences at dose 1, the intersection was outside the threshold dose range of 0-1. For 15/50 at dose 2, incidences 1/50 and 2/50 at dose 1, but not higher, intersected at 0.60 and 0.30 dose units, respectively. For 20/50 at dose 2, thresholds were indicated for incidences up to 4/50, but not for 5/50 (TD's spanning from 0.75 to 0.17 dose units). In addition to the estimation of threshold doses (lower bounds), the procedure allows the estimation of a probability that the true incidences at dose 1 and 2 extrapolate linearly to the incidence in the controls. This could be regarded as a p-value for rejecting the null hypothesis of linearity, for an observed sublinearity.

1080 MULTIPLEX DETECTION OF CYTOKINES AND PHOSPHORYLATED PROTEIN KINASES *IN VIVO* AND *IN VITRO* USING THE BIO-PLEX PROTEIN ARRAY SYSTEM.

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Cytokines and kinases play pivotal roles in cell signaling events, mediating a wide range of physiological responses. Here we present data from the Bio-Plex protein array system demonstrating the sensitive, simultaneous quantitation of 8 cytokines in human or mouse sera, and in tissue culture media. We can quantify all 8 cytokines at a limit of detection less than 10pg/ml, and with inter- and intra-assay CV less than 10%. Using an 8-plex assay, a single 96-well plate can generate 786 concordant cytokine values from 12 µl of sample/well. We also demonstrate a multiplexed sandwich immunoassay that simultaneously measures phosphorylation levels of JNK, p38MAPK, Erk, Akt and IkBa in a single well of a 96-well plate. These high content, multiplexed assays are highly correlated with and as sensitive as ELISA and immunoblotting. All the assays are specific and exhibit no detectable cross-reactivity between antibodies. Most importantly, data obtained from multiplexed cytokine and phosphoprotein assays provide an invaluable profile of cellular response for drug discovery and development.

1081 BIOMARKERS IN HUMAN AND RAT- A PROTEOMICS APPROACH.

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Proteomic technology has demonstrated the potential to increase the predictability of toxicology. Particularly *via* the discovery of biomarkers bridging between animals and man. A study conducted to investigate the effect of Gentamicin on the rat plasma proteome showed that although a large number of proteins displayed changes in expression, a smaller subset showed clear dose or time related changes. These included isoforms of complement components and apolipoprotein. The observed changes in the rat have been compared to protein expression changes in the plasma from Gentamicin treated humans. The studies were conducted as detailed in the table. The changes in protein expression in functionally similar groups of proteins in man and rat are compared in the time course following Gentamicin exposure.

Rat Study	
Doses (mg/kg/day)	0, 0.1, 1.0, 10.0, 40.0, 60.0
Group sizes and study design	Male animals only. 10 rats per treated group, 20 rats in control group. Dosed as above up to day 8, half of each treatment group were sacrificed and tissue and blood samples taken. Surviving animals were treatment free until day 22 and then sacrificed as before.
Blood samples taken	2, 3 and 8 days.
Clinical Chemistry parameters	BUN, creatinine.
Human Study	
Dose	80 mg (iv).
Regime	8 eligible subjects (male) were investigated. Four subjects were randomised to receive placebo (saline iv.) and the remaining 4 subjects received Gentamicin (80 mg iv.) on the first treatment period. On the second treatment period (14 days after the first) they received the alternative.
Blood samples taken	pre dose, 1, 2, 4, 8 12, 24 and 72 hours post dose.
Inulin	Inulin clearance was performed on the day of each admission and approximately 1-2 hours post dose.
Safety assessments	Vital signs, Physical examinations, Medical history updates, 12 Lead ECGs, Clinical Laboratory tests and adverse events were recorded regularly.

1082 RAPID METHOD FOR DETECTION AND IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PROTEINS IN DRUG-TREATED CELLS.

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Cell lines, in response to several drug treatments, regulate the amount of proteins expressed within the cell as well as the secreted proteins to the cell media. We have used a ProteinChip System and the Retentate Chromatography- Mass Spectrometry method (RC-MS) to monitor differentially expressed proteins in the cell media. Many proteins were detected to be up- or down- regulated when proteins in cell media were profiled using several chromatographic ProteinChip Arrays. Two low abundant, small protein biomarkers are subsequently purified for protein identification. Based on their binding characteristics to the arrays, we have quickly developed the protein enrichment and purification using micro spin columns together with a sensitive protein detection method by RC-MS assisted protein purification. We have identified these purified proteins at high confidence after tryptic digestion, peptide-mass mapping on the ProteinChip System and searching the publicly available protein databases. We show this technique as a new method to monitor the toxicological effect of drugs.

1083 IDENTIFICATION OF UBIQUITIN-CONJUGATED PROTEINS USING LC-MS TECHNIQUES: POTENTIAL MOLECULAR INDICATORS OF TOXIC INSULTS.

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Post-translational modification of proteins plays a key role in cellular stress responses and recovery from toxic insult. Ubiquitin, the protein modifier that targets proteins for proteosomal degradation, is now implicated in a number of proteosome-independent signaling processes. In order to study responses in the ubiquitin pathway following intoxication, a method has been developed to screen samples for ubiquitin conjugates. For this work, liquid-chromatography-mass-spectrometry (LC-MS) methods have been combined with newly developed search algorithms to study ubiquitin-conjugated Histone 2A/B. Insoluble fractions were prepared from HEK293 cells and separated by SDS-PAGE. Gel bands of molecular weight 22 kDa, known to contain ubiquitin-tagged Histone 2A and/or 2B, were excised and subjected to in-gel digestion with trypsin. MS-MS spectra were obtained from tryptic peptide mixtures on an LCQ-MS (ion trap). Peptide sequences matching both Histones 2A and 2B, as well as ubiquitin, were independently identified using SEQUEST and SALSA search algorithms to mine LCQ data files. Significant sequence coverage was obtained for Histone 2B (44%) and ubiquitin (34%), and to a lesser extent for Histone 2A (18%). Furthermore, the identification of one particular C-terminal ubiquitin peptide strongly suggests that certain peptides from ubiquitin-conjugated proteins carry a short polypeptide tag specifically identifying the modified residue. Thus far, MS-MS spectra have not yielded sequence coverage for peptides containing the lysine residue specifically modified by ubiquitin, probably because of complications arising from the modification that allow them to evade SEQUEST and SALSA detection. Despite this, Histones 2A and 2B have contributed significantly to our understanding of MS-MS fragmentation characteristics of ubiquitin-conjugated proteins and allowed us to search complex protein pools for molecular indicators of chemical intoxication. (Supported by NIH Grants ES 06694, ES 04940, ES 07091)

1084 IDENTIFICATION OF ORGANOPHOSPHATE INSECTICIDE TARGETS IN SH-SY5Y HUMAN NEUROBLASTOMA CELLS.

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Organophosphate (OP) insecticides increase agricultural production, but are considered highly toxic to humans and animals due to inhibition of acetylcholinesterase (AChE) resulting in cholinergic hyperstimulation. OP's have been implicated in many other pathological diseases, including Gulf War Syndrome, Parkinson's disease, cancers, and various immune dysfunctions. Other esterases, such as neurotoxic esterase, have been shown to be inhibited by OP, but it is likely that OP's interact with and modify the activity of other proteins. We have synthesized a biotinylated fluorophosphonate, FP-biotin, which shares common OP structural characteristics, but has a biotin group attached. Following covalent modification of the protein by FP-biotin, it is possible to visualize or isolate FP-biotin-bound proteins *via* avidin affinity. Our hypothesis is that OP compounds interact with neuronal cell proteins other than AChE. To test this hypothesis, we treated human neuroblastoma cells, SH-SY5Y, with varying concentrations of FP-biotin and tested for AChE inhibition and cell viability over time. Lysates were made of SH-SY5Y cells treated with FP-biotin and biotin-labeled proteins were purified using an avidin-affinity column prior to visualization by western immunobloting. Biotinylated proteins were digested with trypsin either in-solution or in-gel and analyzed by automated nanoLC-MS/MS. Accurate peptide mass fingerprinting combined with peptide fragment analysis was used to identify additional OP-protein conjugates. These experiments demonstrate that OP compounds bind proteins other than AChE in neuronal cells, and may identify complementary pathways to cholinergic hyperstimulation leading to pathology.

1085 UP AND DOWN REGULATION OF PHOSPHORYLATED HEPATIC PROTEINS FOLLOWING ADMINISTRATION OF THE PROTEIN PHOSPHATASE INHIBITOR, MICROCYSTIN-LR TO RATS.

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The field of proteomics is seeing an effort to utilize high throughput methods to quantify the degree in which cellular proteins change when exposed to disease, drugs, toxins, etc. Gallium(III) immobilized affinity chromatography can be used to select phosphorylated peptides. Affinity selected phosphopeptides can be analyzed by using phosphatase enzymes and mass spectrometry. Phosphopeptides are identified by comparing their mass/charge and sequence *via* MS/MS to databases. Quantification of the degree to which peptides and parent proteins are up- or down- regulated is achieved by derivatization with acetate. Control samples are labeled with acetate and experimental samples with trideuteroacetate. Control and experimental samples are mixed, affinity selected and resolved as above. Peptides appear in the mass spectrum as doublets separated by multiples of 3 mass units. The isotope ratio of non-changing proteins is approx. 1:1. Those that have a different ratio have undergone some regulatory change. Microcystin-LR (MCLR) is an inhibitor of protein phosphatases 1 and 2A. However, the change in phosphorylation state is only known for a few proteins which have been specifically identified. In this study, male rats were treated with microcystin-LR (500 ug/kg, ip) or saline. At 6h, they were necropsied. Liver sections were fixed or frozen. Microscopic liver necrosis was typical of MCLR. Frozen liver samples were homogenized, digested with trypsin, affinity-selected *via* gallium-IMAC, derivatized, and quantified *via* mass spec. Ga(III)-IMAC selection captured approximately 2800 phosphorylated peptides. MALDI-TOF mass spec of acetate-labeled peptides identified phosphoproteins which had undergone regulatory change. The majority were up regulated compared to controls, but some were down regulated or unchanged. This suggests that the regulation of protein activity *via* phosphorylation is complex with both increases and decreases in phosphorylation following inhibition of protein phosphatases.

1086 SELDI-TOF MASS SPECTROMETRY PROTEOMIC ANALYSIS OF PYRIDOSTIGMINE BROMIDE AND STRESS ON THE HP-AXIS.

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Pyridostigmine bromide (PB) is an acetycholinesterase (AChE) inhibitor used as a preemptive treatment against possible exposure to organophosphate nerve agents and as a treatment for myasthenia gravis. In order to analyze the effects of PB and stress on the hypothalamic-pituitary axis (HP-axis), SELDI-TOF mass spectrometry proteomic analysis was conducted on the hypothalamus in parallel with AChE activity assays. Two models were used; PB-treatment in which doses of 0, 0.5, 1, 2 and 3 mg/kg/day were administered using Alzet minipumps implanted subcutaneously in the mice for 7 days or 7 day chronic shaker stress. The hypothalamic AChE activity in the 7-day chronic stress model was unchanged when compared to controls. PB (5 mM) was able to inhibit 98% of this activity. In contrast, a significant dose-dependent decrease in hypothalamic AChE activity (>40% at the highest dose of 3 mg/kg/day) was observed in the chronically-treated PB model. Proteomic analysis of the same stress and PB-treated hypothalamic homogenates by SELDI-TOF MS showed significant and similar changes in the hypothalamic peptide/protein profiles for both stressed and PB-treated mice. Ratio analysis of the protein profiles indicated a decrease in peak intensity for large (>4000 d) and small (<4000 d) peptides. However, the decrease in intensity was greater for the larger proteins (2.0-7.5 vs small, 1.3-2.0), suggesting a buildup of the smaller peptides within the cells. Analysis of the posterior pituitary also showed a similar decrease in arg-vasopressin peptide in both stress and PB-treated mice. These results suggest that stress

and PB both affect the peptide protein profiles in the hypothalamus by changing either the protein expression or peptide processing and secretion patterns of the cells. Supported by DoD contract No.99214005.

1087 A PROTEOMIC APPROACH TO THE CHARACTERIZATION OF ARDS.

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Acute Respiratory Distress Syndrome (ARDS) is a severe lung dysfunction associated with a variety of catastrophic events. The condition is characterized by bilateral infiltrates of protein-rich fluid and large numbers of neutrophils. Because ARDS can be induced by multiple factors that produce similar pulmonary pathophysiologic changes, the mechanism likely involves a disturbance of basic homeostatic processes that protect and maintain normal lung permeability. Bronchoalveolar lavage fluid (BALF) contains a large number of proteins that comprise a source for testing and characterizing biochemical alterations associated with airway disease. Proteins that are regulated, modified, secreted or exudated in the airways during disease processes can provide vital mechanistic information, as well as identify novel treatment targets. Our Lab. uses proteomics techniques to identify proteins that are differentially expressed in ARDS by defining protein expression profiles (PEPs) in BALF. Human BALF samples from patients who have undergone bronchoscopy during the progression of their disease have been characterized using 2-DE gels, PDQuest software and Matrix-assisted laser desorption/ionization-time of flight-mass spectrometry. These human PEPs are being used to guide development of an ARDS animal model. Mouse was chosen for the animal model because its genome has the most complete sequence database available for protein identification. In mice we have compared control BALF to BALF from animals with trauma induced acute lung injury. In the trauma model BALF, the PDQuest software identified 30 protein spots that increase by at least 2-fold, 3 spots that decrease by 2-fold, and 14 spots not observed in control BALF. Glutathione-S-transferase and selenium binding protein are two of the up-regulated proteins. Correlation of human data with the mouse data will be used to validate this acute lung injury in mouse as an appropriate animal model of ARDS. Research supported by LRRI.

1088 PROTEIN EXPRESSION IN RAT TESTICULAR TISSUE FOLLOWING MULTIPLE EXPOSURE LEVELS OF JP-8 VAPORS FOR 91 DAYS.

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JP-8 jet fuel is being universally used by the US military. The Navy is primarily concerned about vapor exposures on ships. A 91-day inhalation exposure study was conducted in male Sprague-Dawley rats to evaluate reproductive system toxicity. Testicular tissue was harvested at the necropsy and prepared for proteomic evaluation. 2D electrophoresis and peptide mass fingerprinting procedures were used to generate protein expression profiles. More than 50 proteins were identified as being specific to the testes. These protein spots were excised robotically and identified by the MALDI-TOF-MS system. Increased relative protein abundance was observed in the low dose rats (250 mg/cubic meter). Declines in protein expression were observed in the high dose (1000mg/cubic meter). A total of 49 changes in protein expression differences were observed in the low dose group, 24 differences in protein expression were present in the mid dose group (500 mg/cubic meter), and 26 differences were observed in the high dose group. Specific proteins that showed increased abundance in the low dose group were generally associated with cell repair, and the proteins that demonstrated decreased abundance are generally associated with cell damage. These protein expression patterns may help to understand the mechanism of cellular changes in the testes that occur prior to functional and morphological evidence of male reproductive system functional defects. Proteomics data are being used to fill current reproductive toxicity data gaps in the risk assessment of JP-8 vapor. The information is being used to help formulate appropriate exposure levels for Navy personnel to JP-8.

1089 PROTEIN PROFILING OF RAT LIVER ORGANELLES AFTER SUBACUTE PHENOBARBITAL EXPOSURE:COMPARING PROTEOMICS AND CDNA MICROARRAY DATA.

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Abstract A primary focus of toxicogenomics concerns differential gene expression produced by chemical exposure that form expression signatures that can distinguish toxicant classes and actions. Protein expression was studied in male rat liver by proteomics after subacute exposure to the metabolic enzyme inducer, phenobarbital. Proteins were fractionated into mitochondria, endoplasmic reticulum (ER), cytosol and nuclei, separated by two-dimensional(2D) gel electrophoresis, quantitated by image analysis and identified by mass spectrometry. Over 60 proteins were identified throughout all fractions including the expected induction of cytochrome P450 and glutathione transferase subunits as confirmed by western blot. Several intermediary metabolic enzymes were affected by phenobarbital in cytosolic fractions, among them carbamoyl phosphate synthetase 1, 3 α hydroxysteroid dehydrogenase, arginosuccinate synthase and 10-formyltetrahydrofolate dehydrogenase. Changes observed in nuclear samples include downregulation of LBP-p40, hnRNPA3, hnRNP A2/B1 and hnRNP A1b as well as upregulation of hnRNP L. Results from Western analysis with an LPB40 specific rabbit polyclonal antibody were consistent with the 2D gel results previously observed. In addition, nine potentially new nuclear proteins were found by mass spectrometry not in public genomic databases. This study shows how proteomics might serve to establish protein signature profiles of chemical exposure in toxicogenomics studies and can also serve as an effective tool for gene discovery.

1090 IDENTIFICATION OF POTENTIAL *IN VITRO* ENDPOINTS FOR SKIN IRRITATION USING PROTEOMIC AND MICROARRAY ANALYSIS.

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Understanding the mechanistic basis of the human skin irritation response is key to the development of relevant *in vitro* test systems for the predictive identification of skin irritation hazards. Recent progress in the development of proteomic and microarray technologies means that tools for the identification and investigation of important biochemical events in the processes of skin irritation are now available. This study was designed to identify proteins (and genes encoding proteins) which may be involved in the skin irritation response, following exposure of a reconstructed human skin model (EpiDerm™ (MatTek)) to the skin irritant sodium lauryl sulphate (SLS). EpiDerm™ cultures were treated in triplicate with a non-cytotoxic dose of SLS (0.1mg/ml, as determined by the MTT assay and histological examination) for 15min, 1h, 2h, 4h and 24h. Proteomics was performed using 2D-gel electrophoresis (Multiphor II and DALT system, (APB)) in combination with mass spectroscopy to investigate the protein expression profile and identify proteins of interest. In addition microarray analysis was performed using DermArray (Research Genetics) cDNA arrays covering 5000 genes of relevance to skin biology. 67 proteins of potential interest were selected and identified from a range of 2D-gels (1st dimension : pH 4-7, 4-5, 5-6, 6-9 and 6-11). Of the proteins selected, 35 were upregulated, 19 downregulated and the expression of 4 remained unchanged following exposure to SLS. Data indicated that post-translational modification occurred at an early time point (15 min) for calmodulin-like skin protein and involucrin following exposure of EpiDerm™ to SLS. Epithelial cell marker protein demonstrated post-translational modification after 1h exposure to SLS. These results demonstrate the differential regulation of a number of proteins in response to a known irritant, which could represent potential new *in vitro* markers of skin irritation.

1091 HALOACETIC ACIDS PERTURB PROTEIN PHOSPHORYLATION IN MOUSE EMBRYOS *IN VITRO*.

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Haloacetic Acids (HAAs) formed during the disinfection process are present in drinking water. *in vitro* exposure of rodent conceptuses to these agents produces dysmorphology including craniofacial defects. Based on the effects of HAAs in adult tissues, we tested the hypothesis that exposure to HAAs perturbs signal transduction pathways. CD-1 mouse conceptuses (3-6 somites) were exposed to HAAs at concentrations that produce 100% dysmorphology [11mM dichloroacetate (DCA), 350μM dibromoacetate (DBA) or 300 μM bromochloroacetate (BCA)] in whole embryo culture. To determine if exposure to the HAAs perturbs protein kinase activity, embryos were radiolabelled with 33-P-phosphate in HAA-containing or control medium during 0-2, 4-6 or 22-24 hours of exposure. 2D gel electrophoresis was then performed and autoradiographs compared using Phoretix 2D Software (Nonlinear Dynamics). Both time and chemical changes in protein phosphorylation were observed. To further evaluate protein phosphorylation, Western analysis on 1D gels was conducted using a phospho-tyrosine antibody (PY-99: Santa Cruz Biotechnology). Two major phosphotyrosine containing proteins (122 and 183kDa) were found to be affected by HAA exposure. No changes were observed as early as 2 hours after treatment but an increase in the 122kDa protein was found 6 hours following DCA exposure. By 24 hour after culture both phospho-

proteins were increased in all three HAA treated groups with DCA producing the greatest effect. These changes indicate that the HAAs perturb signal transduction by altering the phosphorylation state of phospho-tyrosine proteins. Since pharmacological protein kinase inhibitors also produce dysmorphology, HAA-induced alteration of signal transduction may be responsible for altered differentiation and development. This abstract does not present EPA policy.

1092 LOCALIZATION OF IKB AND IKK SUBUNITS IN THE SMOOTH ENDOPLASMIC RETICULUM: ACUTE EFFECTS OF TCDD IN MALE AND FEMALE RAT LIVER.

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Effects of acute 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure upon protein expression in rat liver endoplasmic reticulum (ER) were determined by two-dimensional gel separation and mass spectrometry. Liver ER was prepared as microsomes from male and female Sprague-Dawley rats exposed for 72 hr to TCDD. TCDD induced cytochrome P450 enzymes, GST subunits, Hsp72 and Grp78. Mass spectrometry also revealed the presence of increased levels of IKB kinase beta (IKK-β) after dioxin treatment in males and females. This finding led us to search for other NF-κB binding proteins or kinases in ER and possible alterations by TCDD. Western blot for IKB family members in ER showed that IKB-β and IKB-γ were highly abundant in ER while IKB-α and IKB-ε proteins were barely detectable. TCDD induced hyperphosphorylated forms of IKB-β in both genders, but only the 85kd form of IKB-γ was present in females while males expressed an additional IKB-γ immunoreactive form at 115 kD. Although IKK-β was in ER of both genders, IKK-α was the most abundant IKK subunit and IKK-γ was not detectable. The effect of gender and TCDD treatment upon IKB and IKK family member expression was also examined in cytosol for critical comparison to the ER. In cytosol, all IKK subunits were expressed without substantial effect by TCDD. IKB-α, IKB-β, and IKB-ε, but not IKB-γ, were expressed in cytosol and were unaffected by TCDD. Hyperphosphorylated forms of IKB-β were observed in cytosol only in female (not male) rats but were not increased by TCDD as was the case in ER from females. Although NF-κB-p65 was readily found in male and female cytosol, it was not detectable in ER. IKK-α, β kinase activity, measured after immunoprecipitation from cytosol and solubilized ER, was unaffected by gender and treatment. These studies demonstrate a strength of proteomics in showing a unique distribution of NF-κB binding proteins and their kinases in liver ER as affected by gender and TCDD treatment.

1093 T-2 AND HT-2 TOXIN INDUCED APOPTOSIS IN HL-60 HUMAN PROMYELOCYTIC LEUKEMIA CELLS.

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T-2 and HT-2 toxins belong to a group of mycotoxins, trichothecenes, which are widely encountered as natural contaminants. Exposure to such toxins may cause severe damage to the gastrointestinal mucosa and the immune system. In the present study the human promyelocytic cell line HL-60, was used to characterize the apoptotic effects of T-2 and its metabolite HT-2. Apoptotic cells were identified microscopically by chromatin condensation and nuclear fragmentation, by flow cytometric analysis and by DNA laddering. Apoptosis induced in the HL-60 cells following exposure to T-2 and HT-2 for 24 hours was concentration-dependent starting at 3.1 and 6.25 ng/ml, respectively. Little cytotoxicity (membrane damage) was observed even after exposure to concentrations (25-50 ng/ml) that induced 60-100 % of the cells to undergo apoptosis. Following exposure to 6.5 ng/ml an increased amount of apoptotic cells could be observed already after 4-6 hours. Whereas the absolute effects of toxin-induced apoptosis in the HL-60 cells seemed to vary somewhat during culturing and between different batches of cells, T-2 was more potent than HT-2. The apoptotic process could be almost completely blocked by the addition of z-VAD.fmk. In contrast, no or only minor effects were observed by the addition of DEVD.CHO, IETD.fmk and DEVD.fmk. As judged by Western blotting, no or only minor changes in bax and bcl-2 levels were observed, whereas PARP was totally degraded already 3 hours after toxin addition. Furthermore, the level of several procaspases (-3, -7, -8, -9, but not -12) were down regulated. Zn^{2+} and BAPTA-AM blocked the induction of apoptosis as judged by microscopic analysis and flowcytometry, as well as DNA fragmentation. The results suggest that T-2 and HT-2 both cause an increase in the level of Ca^{2+} thereby initiating a signal that activates several caspases resulting in chromosomal condensation, nuclear fragmentation, activation of DNA endonucleases and DNA laddering.

1094 ALTERATIONS IN EXPRESSION OF CYTOKINE NETWORK AND APOPTOSIS SIGNALING GENES IN MOUSE LIVER AFTER SUBACUTE FUMONISIN B_1 EXPOSURE.

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Fumonisin B_1 (FB_1) is a naturally occurring mycotoxin produced primarily by *Fusarium verticillioides* and related fungi, which are common contaminants of corn throughout the world. FB_1 is a carcinogen and causative agent of several lethal animal diseases. Liver is the primary target organ in mice. Our previous studies showed altered expression of cytokines in mouse liver after FB_1 treatment. To further investigate the genes involved in the cytokine network and apoptosis signaling, male and female BALB/c mice (5/group) were injected subcutaneously with either saline or 2.25 mg/kg/day of FB_1 for 5 days. FB_1 treatment caused increased expression of tumor necrosis factor α (TNFα), interleukin (IL)-1α, IL-1β, IL-1 receptor antagonist (IL-1Ra), IL-6, IL-10, IL-12 p40, IL-18 and interferon γ (IFNγ) in male liver, with a similar increase in females except that IL-1β and IL-18 were unaltered. Control females showed higher basal levels of IL-1α, IL-1Ra, IL-10, IL-12 p40 and IFNγ as compared to males. Expression of TNF receptor 55 and TNF receptor associated death domain (TRADD) was increased, with no changes in Fas signaling molecules, Fas, Fas ligand, Fas associated death domain (FADD) and Fas-associated protein factor (FAF). Expression of c-Myc, B-Myc, Max and Mad oncogenic transcription factors and apoptotic genes, namely Bcl-2, Bax, Bad and caspase 3 was also increased after FB_1 treatment. FB_1 caused an activation of cytokine network in liver along with TNFα signaling pathways. FB_1-induced expression of TNFα, IL-1α, IL-1β, IL-6 and IFNγ could play a role in the observed liver toxicity, whereas increased expression of IL-1Ra and oncogenes could be responsible for the cancer promoting properties of FB_1.

1095 IMMUNOTOXIC EFFECTS OF ENDOSULFAN AND PERMETHRIN *VIA* THYMOCYTE APOPTOSIS.

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Altered immune responses have been observed following occupational, inadvertent, or therapeutic exposure to xenobiotics. Many pesticides are known to cause immunotoxicity. Exposure to mixtures of pesticides, either concurrently or sequentially, may result in potentiating this effect mainly because one can effect the metabolism of the other. The objective of this study was to determine the effect the insecticides endosulfan, permethrin and their mixtures on C57BL/6 male mice thymocytes *in vitro*. Permethrin, is a broad-spectrum synthetic pyrethroid, is a widely used insecticide in agriculture and public health. Endosulfan is a highly toxic chlorinated hydrocarbon insecticide used worldwide. We examined the immunotoxic potential of these pesticides using a flow cytometric technique in combination with 7-Amino Actinomycin D (7AAD) to distinguish live, early apoptotic, and late apoptotic/necrotic cells. DNA ladder assay, hallmark of apoptosis, was also used to determine the occurrence of apoptosis. Both endosulfan and permethrin were found to cause significant apoptotic death of thymocytes in a dose- and time- dependent manner. Thus, permethrin at 50, 100 and 300 μ M was found to cause 5.49, 11.49 and 26.11% early apoptotic cell death, respectively. Endosulfan at 25, 50 and 250 μ M was found to cause 11.91, 15.72 and 68.01% early apoptotic cell death, respectively. For the mixture study, concentrations of 100 μ M permethrin and 50 μ M endosulfan was selected and that found to cause 27.09% apoptosis. Thus, these pesticides in mixture have an additive immunotoxic effect. No significant late apoptotic cells were found at these concentrations for either pesticide when exposed for 12 hours. DNA ladder assay confirmed the presence of DNA fragments. The results of this study suggest that the mixtures of endosulfan and permethrin have additive immunotoxic effects on C57BL/6 mice thymocytes.

1096 OXIDIZED PHOSPHATIDYLSERINE STIMULATES PHAGOCYTOSIS OF APOPTOTIC CELLS.

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A critical event during programmed cell death or apoptosis is the acquisition of plasma membrane changes that allows phagocytes to recognize and engulf cells before they rupture. Externalization of phosphatidylserine (PS) has been considered a hallmark of apoptosis. Oxidative stress is an inherent part of the apoptotic program with an unkown specific function. We have previously found that apoptosis induced by oxidants and non-oxidants such as phorbol miristate acetate (PMA) and anti-Fas, was accompanied with PS oxidation and externalization in apoptotic cells. We hypothesize that PS oxidation plays important role in recognition and phagocytosis of apoptotic cells by macrophages. To test this hypothesis, 1-palmitoyl

(C16:0)-2-arachidonoyl (C20:4) PS was oxidized by incubation with 2, 2'-azo-bis-(amidinipropane) hydrochloride (AAPH) and incorporated into plasma membrane of HL-60 cells. By comparing phagocytosis of cells with PS and oxidized PS (PSox) by macrophage J774A.1 cell line, we found that PSox strongly enhanced engulfment of cells by macrophages. Another phospholipid, phosphatidylcholine (PC), when oxidized to the same extent as PS, had no effect on phagocytosis. Liposomes containing PSox inhibited phagocytosis of apoptotic HL-60 cells induced by tert-butyl hydroperoxide(tBuOOH)by macrophages more potent than those containing PS. Based on our experiments with different receptor-specific antibodies, uptake of cells with incorporated PSox was blocked 50% by mAb against both PS-R and Ox-LDL, and 40% by mAb against both CD36 and RGDS. This implies that CD36 and the lectin receptor might be responsible for recognition of PSox while the PS receptor and the scavenger receptor, CD-36, might be involved in recognition of PS. These results suggest that PS oxidation is likely to be a new signaling pathway for clearance of apoptotic cells by macrophages.

1097 VITAMIN E AND RECOGNITON/CLEARANCE OF APOPTOTIC JURKAT CELLS BY MACROPHAGES.

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Phagocytosis of apoptotic cells is important for effective clearance of dead cells without inflammatory reaction. PS externalization, a hallmark of apoptosis, is required for recognition of apoptotic cells by macrophages. Specific PS peroxidation has been documented during apoptosis and suggested to participate in PS-dependent signaling pathways. This implies that antioxidants may affect recognition of apoptotic cells by macrophages *via* inhibition of PS peroxidation. Apoptosis was induced by anti-Fas mAb in Jurkat cells after 48 h culturing with different concentration of alpha-tocopherol acetate (0.25-50μM). Intracellular vitamin E (VE) concentrations were measured by HPLC. PS oxidation was assessed by HPLC after metabolic-labeling of cells with oxidation-sensitive cis-parinaric acid. Apoptosis was assessed by nuclear morphology using Hoescht 33342 staining. For phagocytosis, we used J774A.1 murine macrophages cell line. Percentage of apoptotic cells was 7±1, 11±3, 33±2, 31± 1, 28±3, 35±3 in the control without any treatment, VE without anti-Fas mAb, anti-Fas mAb without VE, and 0.25, 2.5, 50 μM VE plus anti-Fas mAb, respectively. Percentage of phagocytosis-positive macrophages was 5±1, 5±2, 25±3, 34±4, 27±8, 29±4 in control without any treatment, VE without anti-Fas mAb, anti-Fas mAb without VE, and 0.25, 2.5, 50 μM VE plus anti-Fas mAb, respectively. These results show that VE treatment had no significant effect on phagocytosis of apoptotic Jurkat cells. Although PS externalization may be one of the final common pathways of apoptotic program that makes the cells recognizable by macrophages, peroxidation of PS can play a role as an important additional signal for one or more macrophage receptors. It is important to determine whether other lipid- and water-soluble antioxidants may affect phagocytosis of apoptotic cells *via* inhibition of PS peroxidation.

1098 HOW MUCH PHOSPHATIDYLSERINE (PS) IS ENOUGH FOR MACROPHAGES TO RECOGNIZE TARGET APOPTOTIC CELL?

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PS usually confined to the internal leaflet of plasma membrane (PM) is externalized on the cell surface during early apoptosis. Externalized PS (PSex) is required for effective phagocytosis and clearance of apoptotic cells by macrophages (MΦ). Others & we found in normal cells PS asymmetry is not absolute suggesting the amount of PS on cell surface is important for MΦ recognition of dead *vs.* live cells. Therefore, we developed a new sensitive method to quantify the amount of PSex. This method is based on the binding of annexin V conjugated with iron beads to PS. Because the beads have a strong iron EPR signal cells containing PSex can be labeled with annexin V-beads (AV-B) and assayed by EPR spectroscopy. We prepared Jurkat cells with known amounts of added PS on their surface by fusing them with PS-containing liposomes. EPR signal magnitude from AV-B labeled cells linearly depended on the amount of PSex. AV-B labeling was specific due to annexin-PS interaction as confirmed by low EPR signal magnitude from cells labeled with beads alone. We found that normal cells on average contains ~0.04 nmol of endogenous PSex/10^6 cells. To determine the level of PS sufficient for triggering phagocytosis, we prepared cells with different amounts of PSex. Phagocytosis percent as determined by fluorescence microscopy depended non-linearly on the level of PSex. In contrast to linear elevation of PSex, phagocytosis had a pronounced lag-phase at low amounts of PS and increased markedly, when PSex exceeded 0.2 nmol/10^6 cells. EPR signal from cells in which apoptosis was induced by camptothecine revealed that at least 1 and 4 nmol PS/10^6 cells were externalized after 1 and 4 h. Thus, MΦ have a sensitivity threshold for the amount of PS on the surface of target cells. Normal cells contain very low levels of PSex that dramatically increased during apoptosis providing reliable recognition of apoptotic cells by MΦ.

1099 ATTENUATION OF CD95-INDUCED T CELL APOPTOSIS BY INORGANIC MERCURY: COMPARISON BETWEEN THE EFFECTS OF MERCURY AND OTHER GROUP II METALS.

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Mercury (Hg), a metal implicated in systemic autoimmune disease, selectively dysregulates CD95-mediated apoptosis. Such dysregulation may be one mechanism that contributes to the accumulation of autoreactive lymphocytes. We have found that submicromolar Hg concentrations impair CD95 induced apoptosis in Type-I (HUT-78) and Type-II (Jurkat) T cell lines as well as in normal human activated $CD4^+$ T cells. Hg blocked the CD95-induced activation of caspase-8, caspases -3 and -7, as well as the association between CD95 and the signaling adaptor, Fas-associated protein with death domain. Multimerization of CD95 on the cell surface was not affected by Hg. Thus, the Hg sensitive step within the CD95 death pathway localizes to the death inducing signaling complex (DISC). Disruption of DISC formation and the downstream activation of caspases may be a mechanism whereby Hg contributes to autoreactivity. Although no clear linkages between other Group II metals (i.e., cadmium, zinc) and autoreactivity have been established, these metals have been shown to inhibit apoptosis in various cells. Thus, we compared the effects of Cd and Zn with Hg on CD95-induced apoptosis. Using Jurkat cells, the influences of Cd and Zn on CD95-mediated caspase-3 activation were investigated. Caspase-3 is an important effector of apoptosis, as many apoptotic inducers converge at its activation. Caspase-3 enzyme activity was assessed in CD95-agonist stimulated Jurkat cells exposed to Cd (0.05-5 μM)or Zn (5-500 μM). A dose-dependent inhibition of caspase-3 activity was noted for both Cd and Zn; however, unlike Hg we suspect that this caspase-3 inhibition is not selective for the CD95 pathway. Cd and Zn likely inhibit caspase-3 directly. As such, CD95-mediated apoptosis is not selectively inhibited by Cd and Zn inhibition, which may explain why there is limited evidence linking these metals with autoimmune disease. Supported by R21ES10351 and T32ES07026.

1100 BIOLUMINESCENT DETECTION OF P53 *IN VITRO* AND *IN VIVO* IN RESPONSE TO DNA DAMAGING AGENTS.

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We have designed tumor cell-based models with the potential to detect apoptotic activity following exposure to genotoxic agents. p53, a known regulator of apoptotic activity in response to DNA damage, is frequently mutated, absent, or modulated in tumor cells. For this study, a luciferase reporter vector that contained p53-response elements upstream of a minimal TK promoter and the luciferase structural gene (p53RE-luc) was used to detect activation of p53 pathways by bioluminescence. Two human lung carcinoma cell lines, A549 cells (p53wt) and H1299 cells (p53-/-), were transfected with p53RE-luc, treated with doxorubicin or cisplatin. Luciferase activity was measured 24 and 48 hours post-treatment. In A549 cells, doxorubicin (0.01 to 0.30 μM) and cisplatin (0.30 μM to 30 μM) produced dose-related increases in luciferase activity when compared to vehicle-treated cells. As expected, luciferase activity was not induced in treated H1299 cells since these cells do not express p53. These results suggest that the observed response of A549 cells to genotoxic agents was mediated by p53. When H1299 and A549 cells were co-transfected with a p53-expression vector and p53RE-luc, p53 expression was dramatically induced in H1299 cells but not in A549 cells. These findings indicate that A549 cells, which are p53 competent, may modulate p53 activity by post-translational modifications. Western blot analysis showed increased phosphorylation of p53 at Ser15 following doxorubicin treatment. This modification has been associated with increased p53 activity. Athymic mice implanted with these bioluminescent reporter cell lines showed increased p53 activity within 6 hr after doxorubicin treatment, which was similar to the results obtained *in vitro*. These findings suggest that the p53RE-luc reporter may provide a rapid, *in vivo* assay that can be used to detect the activity of DNA damaging agents.

1101 SENSITIZATION OF DRUG-RESISTANT B-LYMPHOMA CELLS TO ARSENITE-INDUCED APOPTOSIS BY PHARMACOLOGICAL MANIPULATION OF MAP KINASE PATHWAYS.

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Trivalent arsenic, or arsenite, is a potent inducer of mitogen-activated protein kinase (MAP) pathways, stress proteins, and apoptosis, and is clinically useful in the treatment of some hematopoietic malignancies. However, the signaling pathways

that regulate the induction of apoptosis by arsenite in hematopoietic cells are only partially known. I studied the activation of MAP kinases by sodium arsenite in a panel of B-cell lines derived from human Burkitt's lymphoma that show marked differences in their susceptibility to apoptosis induction, and investigated the hypothesis that treatments that effectively modulate MAP kinase activity can potentiate apoptosis induction in resistant cells. I found that the apoptosis-sensitive ST486 cell line differed from the apoptosis-resistant EW36 cell line, which over-expresses the Bcl-2 protein, with respect to the requirement for activation of the c-jun N-terminal kinase (JNK) pathway for apoptosis induction. Specifically, the sensitive cell line, ST486, underwent extensive arsenite-induced apoptosis at concentrations below 20 micromolar, which do not activate JNK. In contrast, JNK activation, which occurred only at higher concentrations of arsenite exposure, was required for apoptosis induction in the EW36 cell line. Furthermore, the EW36 cells were substantially sensitized to arsenite-induced apoptosis by exposure to prior, non-lethal hyperthermia. Moreover, pretreatment of EW36 cells with a pharmacological inhibitor of p38 MAP kinase also potentiated arsenite-induced apoptosis, and acted synergistically with hyperthermia to further sensitize these cells. Significantly, the experiments revealed that sensitization of EW36 cells is mediated by activation of the JNK pathway and that pharmacological inhibition of the p38 pathway acts synergistically with hyperthermia to overcome Bcl-2 mediated resistance to apoptosis by prolonging a transient period of JNK phosphorylation that occurs immediately after heat shock treatment. (Supported by NIEHS RO1 ES10815)

1102 FATTY ACID RELEASE AND OXIDATION AS FACTORS IN THE APOPTOSIS INDUCED BY LIPOXYGENASE INHIBITORS.

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MK886, an inhibitor of 5-lipoxygenase activating protein (FLAP), and the general lipoxygenase (LOX) inhibitor nordihydroguaiaretic acid (NDGA), induce apoptosis by mechanisms independent of both lipoxygenase (*J. Biol. Chem.* **273:** 28163 (1998)) and FLAP (*Biochem. J.* **340:** 371 (1999)). FLAP can be considered a fatty acid binding protein (FABP), and we have hypothesized that MK886 can affect fatty acid binding at a number of FABP sites. Such an effect may increase intracellular levels of unbound fatty acids, particularly arachidonic acid (AA), and activate apoptosis signaling pathways either directly or following oxidation. In murine pro-B FL5.12 cells, MK886 (10 μM), baicalein (a LOX inhibitor) (50 μM) and NDGA (10 μM) significantly enhanced the release of [^{3}H]-AA within 2 h and induced apoptosis by 8 h. The presence of peroxides after treatment of FL5.12 cells with these agents was assessed by the ferrous oxidation-xylenol orange assay as well as by loading cells with carboxy-dichlorofluorescin and assessing its oxidation by flow cytometry. Peroxides were evident with both methods 1 h after treatment with MK886 or baicalein, but not NDGA. NDGA did, however, increase the production of glutathione disulfide. Isoprostane formation was measured as an additional index of lipid peroxidation. By both an ELISA and GC-MS assay, isoprostane levels were increased 3-fold 2 h, and 4-fold 4 h after MK886 or baicalein, but not after NDGA. The addition of AA (25-50 μM) directly to FL5.12 cells, induced apoptosis. In addition, pretreatment with PPARγ ligands (ciglitazone, 15δPGJ2, troglitazone) to increase PPAR (another type of fatty acid binding protein) enhanced the toxicity of MK886. These data support the hypothesis that fatty acids are released and oxidized species are generated in cells undergoing apoptosis in response to treatment with MK886, baicalein and NDGA. The time-course of formation of these species is consistent with their having a causal role in apoptosis. (Supported by NIH grant CA83701.)

1103 HUMAN MITOCHONDRIAL THIOREDOXIN (MTTRX) CONFERS RESISTANCE TO OXIDANT-INDUCED APOPTOSIS.

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Oxidant damage to mitochondria is a central mechanism of apoptosis induced by many toxic chemicals. Thioredoxin family proteins share a conserved Cys-X-X-Cys motif at their active center and play important roles in control of cellular redox state and protection against oxidative damage. In addition to the well studied cytosolic and extracellular form (Trx1), a rat mitochondrial form of thioredoxin (mtTrx) has been reported. In this study, we cloned the human mtTrx gene based on available human genome sequencing data and performed localization and functional studies in 143B human osteosarcoma cells. The cloned gene has a putative mitochondrial localization signal (MLS) at its N-terminus. In stably transfected cell lines, mtTrx had a mitochondrial localization as measured by indirect immunostaining and confocal fluorescence microscopy. Subcellular fractionation studies showed that deletion of the MLS rendered it to be solely expressed in the cytosolic fraction. On SDS-PAGE, transfected mtTrx had the same apparent molecular weight as the MLS truncated form, indicating that the leader sequence is cleaved after being imported into mitochondria. Treatment with the oxidant tert-butylhydroperoxide (tBH) induced apoptosis in 143B cells. This oxidant-induced apoptosis was inhibited by overexpression of the full-length mtTrx in 143B cells but not in transfection controls. Thus, human mtTrx functions in protection against oxidant-induced apoptosis. (Supported by NIH grant ES 09047).

1104 OXIDATION AND EXTERNALIZATION OF PHOSPHATIDYLSERINE ON ACTIVATED NEUTROPHIL-LIKE CELLS: ROLE IN APOPTOSIS AND PHAGOCYTIC CLEARANCE.

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To prevent calamitous release of reactive oxygen species (ROS), macrophages remove excess activated neutrophils from an inflammatory site in a regulated way, through processes that ensure swift resolution of inflammation yet make provision for neutrophils to fulfill their microbicidal function. Clearance of activated neutrophils from inflamed areas is achieved *via* their apoptosis whose mechanisms are still poorly understood. In the present work, DMSO-differentiated HL-60 cells were used as a model to study NADPH oxidase-induced apoptosis pathways typical of neutrophils. Activation of NADPH oxidase by phorbol myristate acetate (PMA) caused oxidative stress shown by massive superoxide production and depletion of intracellular glutathione content. Importantly, among the major classes of phospholipids, preferential oxidation of phosphatidylserine (PS) occurred in cells upon PMA stimulation, along with its externalization on the cell surface. Furthermore, PMA stimulation of NADPH oxidase caused apoptosis as evidenced by chromatin condensation and nuclear and DNA fragmentation, and recognition and ingestion of stimulated cells by J774A.1 macrophages. Inhibition of NADPH oxidase by either diphenylene iodonium (DPI) or staurosporine or by scavenging of ROS by SOD/catalase inhibited expression of apoptotic biomarkers and prevented oxidation and externalization of PS. Furthermore, inhibition of NADPH oxidase-induced ROS production reduced recognition and phagocytosis by J774A.1 cells. We conclude that NADPH oxidase-induced oxidative stress in neutrophil-like cells triggers apoptosis and subsequent recognition and removal of apoptotic cells from inflammatory sites through pathways dependent on oxidation and externalization of PS.

1105 CYCLOPENTA POLYCYCLIC AROMATIC HYDROCARBONS INDUCE CYTOCHROME P450 1A1, ACCUMULATION OF CELLS IN S-PHASE AND APOPTOSIS IN MOUSE HEPATOMA (HEPA 1C1C7) CELLS.

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Cyclopenta polycyclic aromatic hydrocarbons (CP-PAH) are formed by incomplete combustion of organic material. The CP-PAH cyclopenta(c, d)pyrene (CPP) has been detected in diesel and automobile exhaust at levels 5-20 times higher than the well known PAH benzo(a)pyrene (B(a)P). CPP has been found to be highly mutagenic in both bacterial and mammalian cells, and to induce tumors in mouse skin and lung. Two others CP-PAH, benz(j)aceanthrylene (B(j)A) and benz(l)aceanthrylene (B(l)A), have been shown to produce lung adenomas and to be potent skin tumor-initiating agents in mice with approximately the same activity as B(a)P. Hepa 1c1c7 cells constitute a powerful experimental system for studying the effects of PAH. Here we show that B(j)A was as potent as B(a)P to induce CYP1A1 in these cells as determined by Western blotting. With CPP only a small increase in the level of CYP1A1 was seen, furthermore CPP also caused an increase in CYP1A2. All the compounds resulted in accumulation of cells in the S-phase. At higher concentrations/later time points an increasing number of dead cells appeared. A major part of these cells was apoptotic judged by microscopy, flow cytometric analysis and DNA laddering. The addition of α-naphthoflavone, an inhibitor of CYP1A1, almost abolished the induced apoptosis and accumulation of cells in S-phase, whereas antioxidants had no or only minor effects. The caspase inhibitor z-VAD.fmk partially reduced the number of apotoptic cells. As judged by Western blotting, B(a)P and the CP-PAH increased the levels of p53 and bax, whereas a decreased level of bcl-2 was seen. Furthermore, the levels of several procaspases (-3, -7, -8) were down regulated. The mitogen-activated protein kinases (MAPKs) also seemed to be involved in the apoptotic process. Both the addition of SB202190 (an inhibitor of p38), and DP98059 (an inhibitor of ERK) reduced the apoptosis and accumulation of cells in S-phase.

1106 P38-MITOGEN-ACTIVATED-PROTEIN-KINASE-MEDIATED BAX TRANSLOCATION INITIATES CYTOCHROME C RELEASE IN CYANIDE-INDUCED APOPTOSIS.

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Cytochrome c release from mitochondria is a critical event in cyanide-induced apoptosis. To determine how cyanide treatment initiated cytochrome c release, we investigated endogenous Bax translocation in primary cultured cortical neurons. Under normal conditions, Bax resided mainly in cytoplasm. After 300 μM cyanide treatment for 1hr, Bax translocated specifically to the mitochondria as shown by immunocytochemical staining and western blotting analysis. Bax translocation preceded cytochrome c release from the mitochondria, which was initiated 3 hr after cyanide treatment. To determine that the translocation of Bax to mitochondria initiated cytochrome c release, double immunofluorescence labeling for both Bax and cytochrome c was used. This study showed that cytochrome c was released only in cells harboring Bax in mitochondria. In cells where Bax translocation did not occur, cytochrome c was still associated with mitochondria. P38 mitogen-activated protein (MAP) kinase was involved in regulating Bax translocation in cyanide-induced apoptosis. This kinase was activated 30 min after cyanide and its phosphorylation began to decrease 3 hr later. SB203580, a specific p38 MAP kinase inhibitor, blocked translocation of Bax to mitochondria. Inhibition of p38 MAP kinase by SB203580 blocked all downstream effects of Bax translocation including cytochrome c release, caspase activation and DNA fragmentation. These results demonstrated that Bax translocation was critical for cyanide-induced cytochrome c release and p38 MAP kinase was involved in regulating Bax translocation from the cytosol to mitochondria. (Supported by NIH grant 04140)

1107 APOPTOTIC EFFECT OF LINOLEIC ACID ON 15LO OVEREXPRESSING ENDOTHELIAL CELLS.

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The development of atherosclerosis is postulated to occur *via* endothelium alterations caused by unsaturated fatty acids and inflammatory cytokines. To test the hypothesis that 15-lipoxygenase (15-LO), a non-heme iron-containing enzyme, causes oxidant injury and apoptosis in endothelial cells the following experiment was conducted. ECV-304 (parental line) and ECV-LO cells (ECV-304 cells stably transfected with human 15-LO) were treated for up to 4 hr with 0, 50, 100, 200 μM linoleic acid (LA) and/or 7.5 μM NDGA, a non-specific inhibitor of lipoxygenase. Apoptosis was monitored by measuring changes in cellular status such as oxidative stress, intracellular calcium homeostasis, F-actin content, nuclear morphology, mitochondrial potential and c-Jun N-terminal kinase (JNK) activity. A 2-fold increase in oxidative stress occurred in ECV-LO compared to ECV-304 cells within 30 min of 200 μM LA. Nuclear fragmentation, decreased mitochondrial potential, and a significant decrease in F-actin content were seen within 15-60 min of 100 μM LA treatment in ECV-LO cells. Similarly, significant decreases in F-actin content and mitochondrial potential were seen in parental cells compared to control. However, these cells also had significant nuclear condensation within 30-60 min of treatment. Pretreatment of ECV-LO cells with NDGA inhibited nuclear fragmentation caused by 100 μM LA, but had no effect on observed decreases in mitochondrial potential or F-actin content. No effect on JNK activity was noted. Significant increases in intracellular calcium occurred in ECV-LO cells within 15 min of LA treatment. These results suggest that LA produces DNA fragmentation and oxidative stress in the endothelium as early as 15 min posttreatment. Mobilization of intracellular calcium may contribute to the apoptotic events observed in these cells.

1108 DMBA-INDUCED PRE-B CELL APOPTOSIS : INTRACELLULAR SIGNALING PATHWAYS.

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The purpose of this study was to investigate intracellular signaling pathways of PAH-mediated, pre-B cell apoptosis and to compare these pathways to those activated in immature B cells. Apoptosis of immature WEHI-231 B cells induced by IgM crossing linking models deletion of autoreactive clones. Dexamethasone(Dex), which also induces B cell apoptosis, has been used to define apoptosis mechanisms in these cells. In both cases, WEHI-231 cell apoptosis is preceded by an increase in expression of $p27^{kip1}$ and $p21^{WAF1}$, growth arrest, NF-κB inactivation, and a reduction in NF-κB-regulated c-Myc expression. All of these changes play a causal role in WEHI-231 growth arrest and apoptosis. Previously, we demonstrated that prototypic PAHs, including DMBA, induce pre-B cell apoptosis in co-cultures of a pre-B cell line(BU-11) and cloned bone marrow stromal cells(BMS2). Similar to system employing immature WEHI-231 cells, DMBA treatment was shown herein to induce down-regulation of NF-κB activity, as assayed in EMSAs with oligonucleotide probes derived from the *c-myc* promoter, and c-Myc expression prior to pre-B cell death. However, unlike the WEHI-231 system, DMBA did not induce pre-B cell growth arrest and did not increase $p27^{kip1}$ or $p21^{WAF1}$ expression. Furthermore, Dex induced pre-B cell growth arrest and c-Myc down-regulation without down-regulating NF-κB or inducing apoptosis. These results indicate that: 1) pre-B cells and immature B cells can undergo apoptosis through distinct mechanisms which may or may not involve $p27^{kip1}$ or $p21^{WAF1}$, 2) growth arrest is not required for apoptosis in all B cell subsets(e.g. DMBA-treated pre-B cells), and 3) NF-κB and c-Myc down-regulation is common to apoptosis pathways activated in immature B cells by surface Ig cross-linking or Dex treatment and to apoptosis pathways initiated in pre-B cells by DMBA treatment. These results help define environmental chemical-induced apoptosis pathways and demonstrate the utility of these chemicals in dissecting apoptosis signaling pathways in normal B lymphocytes.

1109 REGULATION OF APOPTOSIS SENSITIVITY TO MITOCHONDRIAL TOXICANTS AND PESTICIDES BY BCL-2 PROTEIN AND MAP KINASES IN LYMPHOID CELL LINES.

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The toxicity of environmental chemicals towards lymphoid cells appears to involve several components of the apoptotic machinery such as mitochondrial alterations, Bcl-2 proteins, and MAP kinase pathways. However, it remains to be determined how, and to what extent, each of these components influences cellular sensitivity or resistance to chemicals. The present study was designed to examine the relative contributions of Bcl-2 protein expression and MAP kinase signaling to the sensitivity of T-lineage lymphoid cells to chemicals targeting mitochondrial functions. The agents included the uncoupler carbonyl cyanide m-chlorophenylhydrazone (mClCCP) and the respiratory chain inhibitor rotenone (ROT). The herbicide alachlor was included in the study to ascertain whether Bcl-2 proteins may regulate its immunotoxic effects. A Jurkat wild-type (J-wt) cell line and a Jurkat Bcl-2 (J-bcl) stably transfected cell line were exposed for 24 and 48 hr to the three chemicals and assayed for the induction of apoptosis with the DNA-specific fluorochrome Hoechst 33342 and PARP cleavage. Immunoblotting analyses for MAP kinases and c-jun expression were performed at intervals from 2 to 8 hr. Alachlor, mClCCP, and ROT induced apoptosis extensively (50-60%) in J-wt cells but hardly in J-bcl cells. mClCCP failed to activate the p38 and JNK pathways. The constitutive levels of activated ERK (P-ERK) declined in a concentration-dependent manner and with similar kinetics in the two cell lines. ROT activated p38 similarly in the two cell lines, and P-ERK declined similarly with increasing concentration and duration. JNK was activated and c-jun expression preferentially induced in J-bcl cells from 2-8 hr. These results show a strong influence of Bcl-2 protein in the apoptotic resistance of T-lineage lymphoid cells to mitochondrial toxicants and pesticides. Bcl-2 related chemo-resistance is associated with prolonged expression of c-jun. Thus, multiple levels of regulation appear to underlie the chemo-sensitivity and resistance of particular T-lymphoid cell lineages.

1110 SPECIES DIFFERENCES IN THE METABOLISM AND TOXICITY OF 3-BUTENE-1, 2-DIOL.

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3-Butene-1, 2-diol (BDD) is a urinary metabolite of 1, 3-butadiene (BD), a known rodent and human carcinogen. BDD is formed by cytochrome P450-mediated oxidation of BD to butadiene monoxide (BMO) and subsequent hydrolysis of BMO to BDD mediated by epoxide hydrolase. When male B6C3F1 mice were given BDD (250 mg/kg, ip) they excreted approximately 5% of the BDD dose into urine as BDD and its glucuronide and/or sulfate conjugates within 24 h after treatment. These results were confirmed in the present study, which also examined BDD metabolism and toxicity in male Sprague-Dawley rats which are considered less susceptible to BD-induced carcinogenicity in comparison with B6C3F1 mice. Surprisingly, the 250 mg/kg BDD dose was lethal in 2 of the 4 rats given BDD during the 24 h collection period; all of the rats given this BDD dose exhibited hemorrhagic signs. When rats were given a lower BDD dose (125 mg/kg, ip), they did not exhibit any toxicity and there were no alterations in their liver or kidney function tests. Trace amounts of 1-hydroxy-2-butanone, 2-ketobutyric acid, and propionic acid were detected in both mouse and rat urine. However, rats excreted significantly higher amounts of BDD and BDD conjugates in the urine than mice. Because the amounts of BDD and its glucuronide and/or sulfate conjugates recovered in rat urine did not decrease at the low BDD dose, the results suggest saturation of BDD elimination into rat urine at the high dose. Collectively, the results demonstrate significant species differences in the metabolism and toxicity of BDD. The results are also consistent with the previous identification of hydroxymethylvinyl ketone and

1-hydroxy-2-butanone as *in vitro* metabolites of BDD. (Supported by NIEHS grant ES06841. CLS was supported by an Institutional Chemistry-Biology Training grant from NIGMS).

1111 ENANTIOSELECTIVE METABOLISM AND INHIBITION OF GLUTAHIONE TRANSFERASE-ZETA BY BROMOCHLOROACETIC ACID.

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Bromochloroacetic acid (BCA) is a chiral di-haloacetate (di-HAs) that may arise as a drinking water disinfection by-product. Chlorinated and brominated di-HAs are metabolized by glutathione transferase zeta (GSTZ1-1) to either form glyoxylate or cause suicide inactivation of the enzyme. To better understand the molecular events involved in (-), (+) BCA metabolism, we expressed and purified recombinant mouse GSTz and examined the metabolism and suicide substrate qualities of the separate enantiomers of BCA. We also studied the effect of in-vivo and *in vitro* exposure to (-) and (+) BCA on the GSTz activity in hepatic cytosol from male F344 rats. Experiments were also repeated in cytosol obtained from male rats previously treated with dichloroacetic acid to deplete GST-zeta activity. Results using purified GSTz and reduced glutathione as a co-factor indicated only (-) BCA produced glyoxylate. When the enzyme was preincubated with selected di-HAs and glutathione, dialyzed and then assayed with dichloroacetic acid (DCA), both (-)BCA and (+)BCA were found to inactivate the enzyme with similar potency. The inhibition of GSTz by BCA was also shown to be substantially greater than that observed with DCA. Results of cytosol experiments indicated that metabolism of (-)BCA was greatly reduced by modulating GSTz activity in contrast to (+) BCA which was unaffected. Drinking water exposure to (+) BCA concentrations as low as 1.4 mg/l were found to decrease GSTz activity in liver cytotsol. These results indicate (+) BCA is a potent mechanism based inhibitor of GSTz and its metabolism to glyoxylate is controlled by an additional GST isoenzyme as yet uncharacterized. Supported by EPA STAR grant R825954.

1112 ACRYLAMIDE: METABOLISM AND HEMOGLOBIN ADDUCTS FOLLOWING INTRAPERITONEAL, DERMAL, OR INHALATION EXPOSURE.

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Occupational exposure to acrylamide (AM) *via* dermal contact or inhalation may occur during use as an intermediate in the production of polymers, or during the use of AM-containing grouting materials. In this study, AM-derived urinary metabolites and hemoglobin adducts were measured in rats and mice following inhalation exposure and in rats following intraperitoneal (ip) and dermal administration. No mortality occurred from a 6-hr exposure to 5.6 ppm AM-vapor. Rats exposed for 6 hr to 3 ppm [^{13}C /^{14}C]AM-vapor had a lower uptake (90 µmol [^{14}C]AM equiv /kg bw) than mice (245 µmol/kg bw). AM-derived ^{14}C was excreted in 24-hr urine and feces (42% of recovered ^{14}C in rats, 51% mice) or recovered in tissues (56% rats, 46% mice). Following dermal application of 162 mg/kg [^{14}C]AM for 6 hr, 22% of the applied dose was absorbed and distributed to the excreta (44%) or tissues (53%) by 24 hr. Following ip administration of [^{13}C]AM, 62% of the dose was excreted in 0-24-hr urine. In rats, metabolites derived from AM conjugation with glutathione (AM-GSH) accounted for 69% (ip), 52% (dermal), and 64% (inhalation) of the urinary metabolites. In mice, the AM-GSH pathway accounted for only 27% of the urinary metabolites. Remaining metabolites were derived from oxidation of AM to glycidamide (GA). Hemoglobin adducts of AM [*N*-(2-carbamoylethyl)valine, AAVal] and GA [*N*-(2-carbamoyl-2-hydroxyethyl)valine, GAVal] were analyzed by liquid chromatography mass spectrometry. AAVal in rats was 12900 ± 652 (ip), 6398 ± 2979 (dermal), and 1207 ± 127 fmol/mg globin (inhalation), while GAVal was 9031 ± 464 (ip), 10010 ± 3149 (dermal), and 1339 ± 206 fmol/mg globin (inhalation). In mice, AAVal was 1431 ± 509, and GAVal was 4853 ± 564 fmol/mg (inhalation). GAVal:AAVal ratios were 0.7 (i.p. rat), 1.56 (dermal rat), 1.3 (inhalation rat), and 3.4 (inhalation mouse). This study demonstrates marked species and route differences in the metabolism and internal dose (Hb-adducts) of AM and GA. [Funded by the Polyelectrolye Producers Group]

1113 BIOTRANSFORMATION OF 1, 1, 1, 3, 3-PENTAFLUOROPROPANE.

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1, 1, 1, 3, 3-Pentafluoropropane (HFC-245fa) is developed as an alternative to replace chlorofluorocarbons. HFC-245fa has only low toxicity, the only toxic effect seen in rats after inhalation exposure to HFC-245fa in concentrations of up to 50 000 ppm for 90 days was an increased incidence of diffuse myocarditis. To elucidate the role of biotransformation in HFC-245fa toxicity, we studied its biotransformation in rats after inhalation and in rat and human liver microsomes. Rats (n = 5) were exposed to 50 000, 10 000, and 2 000 ppm HFC-245fa for 6 h, urine was collected for 72 h and metabolites excreted were identified by 19F-NMR and quantified by GC/MS. Trifluoroacetic acid and inorganic fluoride were identified as major urinary metabolites of HFC-245fa, 3, 3, 3-trifluoropropanoic acid and 1, 1, 1, 3, 3-pentafluoropropane-2-ol were minor metabolites. After inhalation exposure to 50 000 ppm HFC-245fa, a total of 3 929 ± 441 nmol of trifluoroacetic acid were recovered in urine in males and 3 054 ± 416 nmol in females; trifluoropropanoic acid in urine amounted to 79.3 ± 14.3 nmol in male and 9.1 ± 3.3 nmol in female rats. Extent of HFC-245fa biotransformation after inhalation of 10 000 ppm and 2 000 ppm was linearily dependent on exposure concentrations. In liver microsomes, HFC-245fa was transformed by a cytochrome P450 dependent reaction to trifluoroacetic acid and trifluoropropanoic acid. Rates of formation of trifluoroacetic acid were 99.2 ± 20.5 pmol/mg x min and of trifluoropropanoic acid were 17.5 ± 4.0 pmol/mg x min in rat liver microsomes. In human liver microsomes, rates of formation of trifluoroacetic acid ranged from 0 to 30.4 pmol/mg x min and rates of trifluoropropanoic acid ranged from 0.7 to 7.6 pmol/mg x min. The obtained results suggest that HFC-245fa is metabolized at very low rates *in vivo* and *in vitro*. The toxic effects of HFC-245fa may be due to the formation of the minor metabolite trifluoropropanoic acid, which is highly toxic in rats.

1114 TOXICOKINETICS OF BISPHENOL A IN HUMAN SUBJECTS.

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Bisphenol A is a widely used chemical and is a weak estrogen *in vitro* and in rats *in vivo*. In rats, bisphenol A is only slowly eliminated after oral administration due to enterohepatic circulation of metabolites. To characterize toxicokinetics of bisphenol A in humans, 3 male and 3 female human subjects were orally given 5 mg d16-bisphenol A. d16-Bisphenol A was used due to background concentrations of bisphenol A interfering with quantitation. Urine and blood concentrations of d16-bisphenol A and its glucuronide were determined by GC/MS and LC/MS-MS at different time points. The study was performed with informed consent by the human subjects and approved by the local institutional review board. Free d16-bisphenol A could not be detected in blood and urine samples after d16-bisphenol A administration; the administered dose was completely recovered in urine as d16-bisphenol A glucuronide within 24 h. Bisphenol A glucuronide was cleared by first-order kinetics from blood and with urine with half-lives of < 6 h, no gender differences were observed in maximum blood concentrations and in rates of excretion. The obtained data show that d16-bisphenol A is completely absorbed from the gastrointestinal tract in humans after giving low doses and subject to a complete first-pass metabolism to give d16-bisphenol A glucuronide which is rapidly excreted. The species differences in d16-bisphenol A excretion kinetics between humans and rats are most likely due to the different threshold for biliary elimination. Due to rapid biotransformation to the glucuronide, bioavailability of the weakly estrogenic bisphenol A is very low and accumulation in the human body is not expected due to rapid excretion. This work was supported by Umweltbundesamt, Berlin, Germany.

1115 EXPRESSION AND ACTIVITY OF A NOVEL MUTATION IN THE HUMAN FLAVIN-CONTAINING MONOOXYGENASE 3 GENE.

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Flavin-containing monooxygenases are microsomal drug- and pesticide-metabolizing enzymes implicated in the NADPH-dependant oxidation of a variety of soft nucleophiles. The FMO family comprise 5 isoforms, each demonstrating ontologic, species- and tissue-specific regulation. The major human isoform, liver FMO3, catalyzes the N-oxidation of trimethylamine. Activity of FMO3 is deficient in patients diagnosed with the autosomal recessive human disorder trimethylaminuria. Trimethylaminuria patients excrete relatively large amounts of trimethylamine and results in a fish-like odor of the urine, sweat and breath. Hypertensive crises following consumption of tyramine-containing foods as well as clinical depression have also been observed in patients suffering from trimethylaminuria. As part of a larger effort to establish the frequency of polymorphisms in the FMO family of genes in African-Americans, a novel mutation in FMO3 was found. One individual expressing the mutation demonstrated phenotypic signs of trimethylaminuria, but another did not. The mutation is a GAT to CAT transition at codon 132, resulting in a change from aspartic acid to histidine. The 132 codon is in a region that is well

conserved within the human FMO family. We introduced the mutation into wild-type FMO3 using site-directed mutagenesis and expressed both wild-type and mutated FMO3 as maltose-binding fusion proteins using the pMAL-c2 plasmid and the bacterial JM109 strain. The proteins were purified on an amylose-resin column and the activity of the enzyme was investigated with regards to trimethylamine, 5-DPT (10-[(N, N-Dimethylamino)pentyl]-2-(trifluoromethyl) phenothiazine) and the prototypic FMO3 substrate methimazole. Preliminary activity data indicates that the D132H mutation renders the enzyme virtually inactive.

1116 ROLE OF HUMAN FLAVIN MONOOXYGENASES IN THE SULFOXIDATION OF ALDICARB.

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Biotransformation of the carbamate insecticide, aldicarb, to aldicarb sulfoxide has been shown to significantly elevate its anticholinesterase activity. However, it is unclear what enzymes are responsible for this bioactivation. In this study, 51 human liver microsome samples were prepared and aldicarb sulfoxidation was examined *in vitro* under saturating substrate incubations (200 uM). NADPH-catalyzed sulfoxidation activities were extremely variable and observed in 13 of 51 samples with a mean value of 0.034 +/- 0.17 nmol/min/mg. Coincubation with 0.1% Lubrol to inhibit contributions of cytochrome P450 failed to alter activities (0.036 +/- 0.26 pmol/min/mg). Benzydamine N-oxidation was also examined and was 2.57 +/- 1.66 nmol/min/mg and observed in all samples. FMO3 content was determined in all samples and demonstrated a significant correlation with benzydamine N-oxidation (r2=0.41), but FMO3 did not correlate with aldicarb sulfoxidation. Incubations of aldicarb with 5 recombinant flavin-monooxygenase isoforms indicated catalysis by FMO1>>FMO3 with no activity detected from FMO2, FMO4 or FMO5. These results indicated limited hepatic biotransformation of aldicarb in human liver microsomes, but suggests other tissues may be more important such as fetal liver, the intestine or kidney which possess FMO1.

1117 PHARMACOKINETIC INTERACTIONS BETWEEN PYRIDOSTIGMINE BROMIDE (PB) AND PERMETHRIN IN RATS.

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A single oral dose of 13 mg/kg of PB, and a single dermal dose of 1.3 mg/kg of permethrin, alone and in combinations were applied to male rats. Other animals received an intravenous dose of 1.3 mg/kg of PB or 1.3 mg/kg of permethrin. Five rats were sacrificed at: 0.5, 1, 2, 4, 8, 24, 48, and 72 h after dosing. In rats treated with a single agent, 0.5 and 72 h after dosing, the application site retained 95% and 12% of PB, and 62% and 4% of permethrin, respectively. PB was not detected in the brain. Plasma peak concentrations were detected within 2 h for PB (1, 326 ng/ml), and within 24 h for permethrin (193 ng/ml). Bioavailability of PB, and permethrin was 28%, and 52%, respectively. N-methyl-3-hydroxypyridinium bromide a metabolite of PB was detected in plasma after 2 hr. Permethrin metabolites m-phenoxybenzyl alcohol, and m-phenoxybenzoic acid were identified in plasma, liver and kidney 24 h after administration. In urine, PB and its metabolite N-methyl-3-hydroxypyridinium bromide were identified. Urine contained m-phenoxybenzyl alcohol and m-phenoxybenzoic acid following application of permethrin. A total 30% of urinary excretion of permethrin was in the form of glucuronide and sulfate conjugates. Time-plasma concentration curves for PB and permethrin were fitted into a two and one compartment pharmacokinetic model, with a terminal half-life of elimination of 3.2 h, and 32.6 h for PB, and permethrin, respectively. Following combined intervenous dose of PB and permethrin, PB significantly increased Cmax, AUCplasma, T1/2, and decreased concentration of urinary metabolites of permethrin by 40% and 32%, 10%, and 22% respectively. Also, Cmax, AUCplasma of permethrin was significantly increased (26%, 30%). The results show that PB significantly altered the pharmacokinetic profile of permethrin through interfering with its metabolism and excretion. This is could be due to competition for metabolism enzymes. This work was supported in part by the US army Medical Research and Materiel Command under Contract DAMD 17-99-1-9020.

1118 COMPARISON OF RATES OF GLUTATHIONE (GSH)-CONJUGATION OF TRIHALOMETHANES.

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Investigation into the mutagenic potencies of trihalomethanes (THMs; a class of water disinfection-by-products), mediated by GSH S-transferase (GST) theta, have described an association between a THM's bacterial mutagenicity and its degree of bromination. Tested halomethanes ranked as follows in mutagenic potency: $ClCHBr_2 \approx CHBr_3 >> BrCHCl_2 \approx CH_2Cl_2$. $CHCl_3$ was not mutagenic at doses comparable to mutagenic doses of $BrCHCl_2$. To enable a mechanistic understanding of these effects, we have characterized GSH conjugation rates to compare this data with bacterial genotoxicity results. Several *in vitro* kinetic assays have been performed using THMs, CH_2Br_2 or CH_2Cl_2 and GST theta 1-1. Using a gas-phase equilibration technique, loss of parent compound from the head-space (determined by GC-ECD) did not reveal differences between enzymatic and non-enzymatic rates for each THM; nor were differences in conjugation rates found when different THMs were compared. Similarly, no apparent rate trends were observed among the THMs when formaldehyde (HCHO) production was measured by the Nash assay; in contrast, CH_2Br_2 produced significant amounts of HCHO at equivalent concentrations. A more sensitive coupled assay that indirectly measures HCHO formation resulted in detectable rates for the following THMs (units: nmol•min^{-1}•mg protein^{-1}•mM THM^{-1}): $BrCHCl_2$ (0.22), $ClCHBr_2$ (0.23), $CHCl_3$ (0.05), $CHBr_3$ (<0.05). HCHO does not appear to be responsible for the THM-mediated bacterial mutagenicity as rates of HCHO formation from $BrCHCl_2$ and $ClCHBr_2$ were not different, while the HCHO formation rate from CH_2Cl_2 was ~20-fold greater than for $BrCHCl_2$. That CH_2Cl_2 and $BrCHCl_2$ are equally potent in terms of mutagenicity, yet have significantly different rates of GSH conjugation and HCHO production, argues for existence of two separate pathways responsible for bacterial genotoxicity of di- and tri-halomethanes; suggesting THM-derived intermediates are more DNA-reactive and/or produce adducts that are more mutagenic. (Abstract does not reflect EPA policy).

1119 7H-BENZO[c]FLUORENE *IN VITRO* METABOLISM.

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BENZO(C)FLUORENE (B[c]F) is tumorigenic in lung when administered to A/J mice. The mechanism by which B[c]F induces lung tumors is currently unknown, however, it is likely that the formation of B[c]F:DNA adducts play an important role in lung tumor induction. This study evaluates the *in vitro* metabolism of B[c]F in order to better understand the ability of B[c]F to form DNA adducts. A standard *in vitro* metabolism system using Aroclor induced rat liver S9 was employed. Metabolites were isolated using exhaustive ethyl acetate extraction and subsequently analyzed by reverse phase HPLC equipped with UV detection. The effect of reaction time and B[c]F substrate concentration were also evaluated. The 7-hydroxyB[c]F, 3-hydroxyB[c]F, and the 3, 4-dihydrodiolB[c]F metabolites were synthesized and used to characterize the oxidation products observed with B[c]F. At least 7 oxidation products of B[c]F were detected within a 60 minute reaction time. The HPLC retention time of 7-hydroxy B[c]F corresponded to a predominant B[c]F oxidation product. When reaction times were increased to 160 min this metabolite peak was further converted to a more polar derivative. These results demonstrate that B[c]F is metabolized to a range of oxidation products *in vitro* and that the 7-hydroxy B[c]F metabolite can be further converted to a more polar derivative. These results, combined with recent DNA binding studies indicating that 7-hydroxy B[c]F is limited in its ability to form DNA adducts when applied to mouse skin. This suggests that the formation of 7-hydroxyB[c]F is a detoxification product of B[c]F. (This research was supported funds from EPRI.)

1120 MECHANISM(S) OF DIFFERENTIAL INHIBITION OF HEPATIC AND PANCREATIC FATTY ACID ETHYL ESTER SYNTHASE BY INHIBITORS OF BETA-ESTERASES.

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In previous studies, we reported that hepatic fatty acid ethyl ester synthase (FAEES) activity compared to pancreatic FAEES is inhibited by tri-o-tolylphosphate (TOTP), and that metabolism of TOTP is prerequisite for inhibition of FAEES as well as esterase activity. We also found that FAEES/esterase activity is associated with carboxylesterase (CE) in the liver and cholesterol esterase (ChE) in the pancreas. To further understand the interrelationship between hepatic and pancreatic FAEES, we synthesized two active metabolites of TOTP, 2-o-(cresyl)-4H-1;3;2-benzodioxaphosphoran-2-one (CBDP; cyclic saligenin phosphate) and di-o-tolyl-o-(alpha-hydroxy) tolylphosphate (hydroxylated-TOTP). Inhibitory effect of CBDP and hydroxylated-TOTP were studied using rat post nuclear (PN) fractions of hepatic and pancreatic homogenates, and commercially available porcine hepatic CE and pancreatic ChE. FAEES as well as esterase activity of hepatic PN fraction, and commercial CE and ChE was inhibited by CBDP and hydroxylated TOTP in a dose- dependent manner using 0.01, 0.1 and 1 mg/ml concentrations. No inhibition of the FAEES/esterase activity was observed in the pancreatic PN fraction after

exposure to either TOTP metabolites. Both the activities in the hepatic PN fraction and commercial CE and ChE preparations by TOTP metabolites were found irreversible. Although, the differential effect of the TOTP metabolites on hepatic and pancreatic FAEES/esterase activity is in agreement with the results of our earlier *in vivo* and *in vitro* studies, the anomaly of the differential effect on pancreatic FAEES/esterase activity in the PN fraction and commercial ChE preparation by either metabolite is not understood. Therefore, further studies are in progress on identification of covalent adducts of FAEES and metabolic products of CBDP and hydroxylated-TOTP.

1121 HEPATIC GLUTATHIONE (GSH) CONJUGATION OF COUMARIN 3, 4-EPOXIDE (CE) IN MICE, RATS AND HUMANS.

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CE, a reactive metabolite of coumarin, is thought to mediate coumarin-induced hepatotoxicity in rats. In the absence of GSH, CE spontaneously rearranges to *o*-hydroxyphenylacetaldehyde (*o*-HPA), which is also hepatotoxic. Metabolic activation to CE is highest in mice, a species that shows no evidence of hepatotoxicity, thereby suggesting that detoxification reactions are likely to determine species differences in liver toxicity. The purpose of the present work was to characterize hepatic GSH conjugation of CE in rats, mice and humans. The GSH conjugate (CE-SG), formed at the 3-position, was isolated and used as a standard in an HPLC assay to quantify CE-SG. Non-enzymatic GSH conjugation was observed, accounting for approximately 45 and 80% of total CE-SG formed in mouse and rat liver, respectively. Glutathione S-tranferase (GST)-catalyzed formation of CE-SG was observed in rat and mouse liver cytosols, with the Vmax for this reaction slightly higher in mice than rats (2.3 nmol/min/mg and 1.7 nmol/min/mg, respectively). Rat and human recombinant GST enzymes of the alpha and mu class catalyzed formation of CE-SG. The rat A1-3 and human A1-1 had the lowest Km values, while the highest activities were observed with the rat M2-2 and human M1-1. However, no GST-catalyzed formation of CE-SG was observed with human liver cytosols, suggesting that the formation of *o*-HPA is favored over CE-SG in humans. These results suggest that detoxification of CE by GSH conjugation is not the major determinant of species sensitivity to coumarin hepatotoxicity and implicates the detoxification of *o*-HPA as the critical detoxification pathway.

1122 INFLUENCE OF MATRIGEL-OVERLAY ON CONSTITUTIVE AND INDUCIBLE EXPRESSION OF 9 GENES ENCODING DRUG METABOLIZING ENZYMES IN PRIMARY HUMAN HEPATOCYTES.

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Previous studies have shown that rodent hepatocytes overlaid with matrigel maintain expression of drug metabolizing enzymes, and restore the *in vivo*-like induction response of cytochromes P450 (CYPs). However, whether matrigel provides similar advantages for xenobiotic metabolism studies in primary human hepatocytes is uncertain. Therefore, this study evaluated the influence of matrigel-overlay on the constitutive, phenobarbital (PB) and oltipraz (OPZ)-inducible gene expression in primary human hepatocytes. Hepatocytes were isolated from 5 viable human livers that were rejected for transplantation for various reasons. Hepatocytes were maintained in hormone-supplemented William's E medium on a rigid collagen substratum overlaid either with matrigel or culture media alone, and treated for 48 h with 0.5 and 2 mM PB and 10 and 50 uM OPZ. The mRNA levels of 4 CYPs (1A1, 1A2, 2B6, 3A4) and 5 glutathione S-transferases (GSTs; A1, A2, M1, P1, T1) were determined by real-time RT-PCR. Protein levels of selected enzymes were assessed by immunoblot and activity analyses. Following PB treatment, mRNA expression of CYP2B6, 3A4, GSTA1/2 was increased; OPZ induced mRNA levels of CYP1A2, 2B6, GSTA1/2. Our findings demonstrate further that, in the majority of cases, human hepatocytes overlaid with matrigel showed markedly higher constitutive and inducible gene expression relative to hepatocytes with no matrigel. Furthermore, the constitutive expression of most of the genes in hepatocytes overlaid with matrigel was similar to those in corresponding fresh liver tissue, and higher than hepatocytes with no matrigel. These results demonstrate that matrigel overlay facilitates the maintenance and induction of xenobiotic metabolizing enzymes in primary cultures of human isolated hepatocytes. (Supported by Hochschulsonderprogramm III (DAAD) and NIH grants R01 ES-05780 and P30 ES 07033)

1123 MODELING GENETIC POLYMORPHISMS OF ALCOHOL DEHYDROGENASE.

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The family of cytosolic enzymes known as alcohol dehydrogenase (EC 1.1.1.1) catalyze the reversible oxidation of ethanol to acetaldehyde, with corresponding reduction of NAD^+ to NADH. Alcohol dehydrogenase in humans exists as multiple forms, including class and isozyme multiplicity, as well as allelic variations within several different isozyme forms. In the current study continuous system models were constructed to simulate the activities of those forms of alcohol dehydrogenase that play a major role in the biotransformation of ethanol. Kinetic macroconstants reported in the literature were utilized to calculate microconstants descriptive of the Theorell-Chance mechanism for alcohol dehydrogenase homodimers. Models for the C1 and C2 homodimers, and the B1 homodimer included the previously reported negative cooperativity and substrate inhibition, respectively. Heterodimers were modeled as the sum of the activity of the respective monomers. All models indicated that under equilibrium conditions the reaction favored markedly the reduction of acetaldehyde to ethanol, as has been reported in the literature. In addition, comparisons of simulation output with published data indicated that the models accurately simulated the *in vitro* metabolism of ethanol by alcohol dehydrogenase. Interestingly, sensitivity analyses indicated that dissociation of NADH from alcohol dehydrogenase is not always the rate limiting step for the biotransformation of ethanol. These studies represent the first step in the investigation of how variability from genetic polymorphisms can be incorporated into biologically based models useful for risk assessment. (Supported by a grant from The American Chemistry Council).

1124 METABOLISM OF MENTHOFURAN IN F344 RATS.

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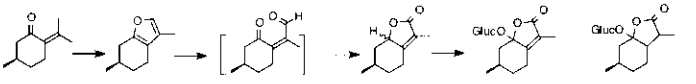
Menthofuran is a major liver microsomal metabolite of pulegone, the chief component of pennyroyal oil. It has been detected in plasma of rats after i.p. administration of pulegone. Up to half of the pulegone hepatotoxicity has been estimated to arise from menthofuran. Menthofuran is further metabolized by liver microsomes to mintlactones through a reactive enonal that binds to proteins. However, previous metabolism studies on pulegone and menthofuran in rats did not report common urinary metabolites derived from this pathway. Recently, we have identified previously unknown phase II urinary metabolites of pulegone. In order to determine the pulegone metabolites derived from menthofuran, a study of ^{14}C-labeled menthofuran in F344 rats at a 60 mg/kg dose was conducted. Urine samples (24 h) were separated by HPLC equipped with a photodiode array detector and analyzed by MS. The urinary metabolite profile of menthofuran is complex. Two of the major metabolites were identified as 7a-hydroxy-3, 6-dimethyl-5, 6, 7, 7a-tetrahydro-2(4H)-benzofuranone (E3) and 7a-hydroxy-3, 6-dimethyl-3, 4, 5, 6, 7, 7a-tetrahydro-2-benzofuranone (G2) through comparison of their molecular weights, HPLC retention times, and UV absorption with those of the corresponding urinary metabolites of pulegone. Identification of common urinary metabolites from menthofuran and pulegone provides evidence for the *in vivo* metabolism of pulegone to menthofuran, then to mintlactones.



1125 *IN VITRO* METABOLISM OF CARBARYL BY HUMAN LIVER MICROSOMES AND HUMAN CYTOCHROME P450 ISOFORMS.

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Carbaryl is a widely used carbamate pesticide. Its effect as a pesticide results from anticholinesterase activity. The present study was designed to investigate the metabolic activity of human liver microsomes (HLM) and human cytochrome P450 (CYP) isoforms toward carbaryl *in vitro*. Carbaryl was incubated with HLM or a CYP isoform at 37°C for 15 min. The 3 major metabolites, i.e. 5-hydroxy carbaryl, 4 hydroxy carbaryl and carbaryl methylol, were analyzed by HPLC. The Km and Vmax for 5-hydroxy carbaryl in HLM were 351 μM and 0.04 nmol/mg protein/min, for 4-hydroxy carbaryl were 421 μM and 0.9 nmol/mg protein/min, for carbaryl methylol were 72 μM and 0.53 nmol/mg protein/min. Using the same experimental conditions, the Km and Vmax in rat liver microsomes were 96 μM and

0.6 nmol/mg protein/min for 5-hydroxy carbaryl, 111 μM and 2.3 nmol/mg protein/min for 4-hydroxy carbaryl, 37 μM and 2.6 nmol/mg protein/min for carbaryl methylol. Metabolic activity toward carbaryl was also investigated in 16 human CYP isoforms. Isoforms with the greatest ability to form 5-hydroxy carbaryl were CYP1A1, 1A2, and 3A4, while the 4-hydroxy carbaryl was formed predominately by CYP3A4, 1A1, and 2C9*1. The carbaryl methylol metabolite was primarily the result of metabolism by CYP2B6, although 1A2 and 2C19 were also involved. The information on CYP isoform activity could be used to estimate differential activity in the human population toward carbaryl and to understand the potential role of biotransformation in interactions of carbaryl with other pesticides or chemicals. (Supported by NCDA Environmental Trust Fund)

1126 INACTIVATION OF SEVERAL HUMAN GLUTATHIONE S-TRANSFERASE ISOFORMS BY METABOLITES OF ESTRADIOL.

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A link has been established between lifetime dose of estrogen and risk of several human cancers such as breast and uterine cancer. In addition to estradiol's putative role as a tumor promoter through interaction with estrogen receptor, estradiol can be metabolized to redox active and/or mutagenic semiquinones and quinones. Furthermore, metabolites of equine estrogens (components of certain hormone replacement therapy preparations) have recently been shown to inhibit human glutathione S-transferases (hGSTs). The purpose of this study was to determine whether this inhibitory effect is unique to equine estrogens or whether it extends to estrogen metabolites found endogenously in humans. The quinone form of 2-hydroxy-17-β-estradiol was synthesized, and inhibition of hGST-mediated metabolism of 1-chloro-2, 4-dinitrobenzene was assessed. Bacterially expressed and purified recombinant hGSTA1-1, hGSTP1-1, hGSTM1-1 and hGSTM2-2 were utilized in this study. Inhibition of hGSTM1-1 and hGSTA1-1 occurred in a concentration-dependent manner with IC_{50}-values of approximately 250 nM and 350 nM, respectively. Interestingly, hGSTM2-2 was significantly less sensitive to inhibition, exhibiting an IC_{50}-value at least 1 order of magnitude greater than hGSTM1-1. hGSTP1-1 was moderately sensitive to inhibition with an approximate IC_{50}-value of 1.1 μM. Specific, spontaneously formed glutathione-conjugates of the estrogen quinone are likely responsible for a majority of the observed inhibition. These data suggest that endogenously-occurring metabolites of estradiol may modulate hGST functions such as toxicant biotransformation.

1127 HUMAN LUNG CELL CYTOSOLS CATALYZE THE BIOACTIVATION OF THE TOBACCO-SPECIFIC NITROSAMINE 4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE (NNK).

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Bioactivation of the potent tobacco-specific carcinogen NNK involves α-carbon hydroxylation that could be catalyzed by cytochrome P450, hemoglobin (Hb), peroxidases and lipoxygenases (LOXs). We have previously observed NNK biotransformation in human lung cytosols incubated with [5-^{3}H]NNK, suggesting a role for Hb, LOXs and/or peroxidases. The objective of this study was to identify the enzyme system(s) responsible for NNK bioactivation in human lung cytosols. Lung cytosols (100 000 g supernatants) were prepared from six human lung specimens obtained during clinically indicated lobectomy. NNK bioactivation to keto acid, determined by reverse phase HPLC and radioflow detection, was observed in human lung cytosols incubated with 4.2 μM NNK (N=6). Keto acid formation in cytosols was inhibited by 37±23 % by 1-aminobenzotriazole (5.0 mM, N=5), a known P450 inhibitor. However, α-methylene hydroxylation was not inhibited by LOX inhibitor nordihydroguaiaretic acid (NDGA, 10 μM) (N=5). Arachidonic acid (AA, 100 μM) also inhibited NNK bioactivation to the keto acid by 22±5.0 % in the cytosols (N=4). Human lung cytosolic LOX was enriched by concanavalin A affinity chromatography. The two activities of LOX, dioxygenase and peroxidase, were quantified in LOX-enriched cytosols (N=6) using published assays and were inhibited by NDGA (10 μM). NNK bioactivation, in particular α-methylene hydroxylation, was not detectable in LOX-enriched cytosols (N=4), even in the presence of exogenous AA, as would be predicted if LOX is active in NNK metabolism. NNK metabolism by purified human myeloperoxidase was not detectable. However, α-methyl hydroxylation of NNK to keto alcohol was observed when NNK was incubated with horse radish peroxidase. These results suggest that NNK bioactivation in human lung cytosols is probably catalyzed by hemoglobin and that LOXs and myeloperoxidase are not involved in NNK metabolism (Supported by CIHR #10382).

1128 THIODIGLYCOL METABOLISM BY ALCOHOL DEHYDROGENASE USING NMR: TWO-STEP OXIDATION PRODUCES 2-HYDROXYETHYLTHIOACETIC ACID AND 2 MOLES OF NADH.

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Sulfur mustard is a vesicant causing delayed injury to human skin. In aqueous medium mustard undergoes rearrangement and hydrolysis to form 2, 2'-bis hydroxyethyl sulfide (TDG), a dihydric alcohol. TDG is able to inhibit protein phosphatases in cytosolic preparations but not the pure enzymes. Therefore, we are investigating metabolic activation. We demonstrated the metabolism of the compound by alcohol dehydrogenases (ADH) from horse liver and human skin [Brimfield et al. 1998. J.Biochem. Molec. Toxicol. 12:361] and from cloned human ADH isoforms [Dudley, Brimfield and Winston. 2000. J. Biochem. Molec. Toxicol. 14:244] by monitoring NAD reduction using OD 340. That technique, however, provides little information about intermediates and products. Recent work with ADH using NMR showed the mechanism varies with increasing substrate chain length and that the mechanistic difference is transparent to spectrophotometry [Oppenheimer and Hennahan. 1993. Adv. Expt. Biol. Med.. 372: 407]. We used Oppenheimer's NMR technique to study the fate of TDG acted upon by horse liver ADH. Work was performed on a Varian 600 MHz NMR. Samples were prepared in 0.1 M $NaPO_4$, pH 7.5 containing 10% D_2O. Spectra were obtained at 37°C at 8 min intervals. The data showed the appearance of a singlet at 3.19 ppm from the methylene protons on carbon 2 of the acetate. Integration provided concentrations of the reactants which allowed us to evaluate kinetics and stoichiometry. The results indicate that TDG is oxidized to 2-hydroxyethylthioacetic acid by horse liver ADH *via* a two-step process in which TDG remains in the active site while two moles of NAD are sequentially reduced. There was no release of intermediate aldehyde as evidenced by the absence of a signal from an aldehydic proton at low field, nor was the monoacid a substrate. The structure was comfirmed by synthesis. Additional metabolism work awaits the availability of cloned human ADH isoforms.

1129 SPECIES VARIATION IN THE METABOLISM, BIOACTIVATION AND CYTOTOXICITY OF CARBARYL *IN VITRO*.

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Pesticides are, by their very nature, toxic. However, the metabolism of these chemicals to reactive metabolites may play a pivotal role in the pathogenesis of chemical toxicity. We have investigated the metabolism, bioactivation and cytotoxicity of carbaryl using human mononuclear leukocytes (MNLs) as targets for toxicity and hepatic microsomes from sheep, chickens and humans as the metabolite generating systems. MNLs, isolated from whole blood, were incubated with hepatic microsomes and [^{14}C]-Carbaryl (3-100μM) for 2h at 37°C in the presence and absence of cofactors. Following incubation stable metabolites were assessed by radio-HPLC-MS, protein-reactive metabolites assessed by exhaustive solvent extraction of the microsomal protein and cell death assessed by trypan blue exclusion. In the absence of NADPH, carbaryl was, in part, hydrolysed to yield 1-naphthol. Protein-reactive metabolite formation was low, however direct cytotoxicity to human MNLs was evident. In the presence of NADPH there was a significant increase in both the rate and routes of metabolism. The rank order of metabolite formation was chicken>sheep> man. The metabolite profile in all species was similar, with the exception that 1, 5 di-OH and 5, 6 di-OH naphthalene were produced by chicken microsomes only. Significant bioactivation of carbaryl was also evident, the rank order of protein reactive metabolite formation being sheep>man>chicken. Cytotoxicity was also increased in the presence of NADPH, with the rank order of cytotoxic metabolite formation being sheep>man>chicken. The introduction of the biologically ubiquitous cytoprotectant, reduced glutathione, decreased both the bioactivation and cytotoxicity, whilst the anti-oxidant, ascorbic acid, decreased the cytotoxicity. In conclusion, hepatic microsomal metabolism of carbaryl may enhance its bioactivation and cytotoxicity. Hepatic microsomes from sheep, chickens and humans, species that may become exposed to this chemical, have been shown to catalyse these biotransformations.

1130 *IN VITRO* METABOLISM OF DIETHYL TOLUAMIDE (DEET) BY HUMAN, MOUSE, AND RAT LIVER MICROSOMES, HUMAN CYTOCHROME P450 ISOFORMS, AND INTERACTION OF DEET WITH CHRLORPYRIFOS.

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Metabolism of the insect repellent N, N-diethyl-m-toluamide (DEET) by pooled human liver microsomes (HLM), rat liver microsomes (RLM), and mouse liver microsomes (MLM) was investigated. DEET is metabolized by cytochrome P450s (CYP) leading to the production of a ring methyl oxidation product, N, N-diethyl-m-hydroxylmethylbenzamide (BALC) and an N-deethylated product, N-ethyl-m-toluamide (ET). Both the affinities and intrinsic clearance of HLM for ring hydroxylation are greater than those for N-deethylation. Pooled HLM show significantly lower affinities (Km) than RLM for metabolism of DEET to either of the primary metabolites (BALC and ET). Among 15 cDNA-expressed CYP enzymes examined, CYP1A2, 2B6, 2D6*1 (Val374), and 2E1 metabolized DEET to the BALC metabolite while CYP3A4, 3A5, 2A6, and 2C19 produced the ET metabolite. CYP2B6 is the principle cytochrome P450 involved in the metabolism of DEET to its major BALC metabolite while CYP2C19 had the greatest activity for the formation of the ET metabolite. Use of phenotyped HLM demonstrated that individuals with high levels of CYP2B6, 3A4, 2C19, and 2A6 have the greatest potential to metabolize DEET. Mice treated with DEET demonstrated induced levels of the CYP2B family, increased hydroxylation, and a 2.4 fold increase in the metabolism of chlorpyrifos to chlorpyrifos-oxon, a potent anticholinesterase. Preincubation of human CYP2B6 with chlorpyrifos completely inhibited the metabolism of DEET. (This research was supported by US Army Cooperative Agreement DAMD 17-00-2-0008)

1131 CHARACTERIZATION OF CATECHIN GLUCURONIDATION IN MICE.

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(–)-Epigallocatechin (EGC) and (–)-epigallocatechin gallate (EGCG) are major tea flavonoids with significant antioxidant and anticancer activities. After intestinal absorption, most of the EGC was in glucuronide form in the plasma. One O-glucuronide of EGC (M21), identified by LC/MS/MS, was predominantly found in human and mouse urine after ingestion of green tea. M21 was synthesized in preparative scale with mouse liver microsomes (MLM). Purified M21 and another EGC-O-glucuronide M16 were characterized by NMR as (–)-EGC-3'-O-ß-glucuronide and (–)-EGC-7-O-ß-glucuronide, respectively. M21 and M16 had very similar kinetics in regard to Km and Vmax although M21 was the predominant form of EGC glucuronides in mouse plasma and urine. The catalytic efficiency (Vmax/Km) of EGC glucuronidation by MLM was 0.74 and 0.65 for M16 and M21, respectively. The two major glucuronidation products of 4'-O-methyl-EGC, the major methylated EGC *in vivo*, had significantly different enzyme kinetics, indicating that the methylation of EGC affects its glucuronidation in site-dependent way. M21 was much more stable than EGC and M16 at pH 7.4. M21 was readily hydrolyzed by E. coli beta-glucuronidase and was weak substrate for hepatic beta-glucuronidase. Two major EGCG glucuronides (M37 and M40) formed by MLM and one major EGCG glucuronide M40 by mouse small intestine microsomes (MSIM) were identified by LC/MS/MS. The catalytic efficiency of EGCG glucuronidation by MLM was 0.18 and 0.35 for M37 and M40, respectively. Interestingly, The catalytic efficiency of EGCG glucuronidation by MSIM was 0.61 for M40, which is higher than that by MLM and close to that of EGC glucuronidation by MLM. After 0.6% green tea treatment for 16 days, the Vmax for M21 and M16 in MLM increased significantly whereas the Vmax for EGCG glucuronidation by MSIM decreased dramatically.

1132 *IN VITRO* DETERMINATION OF METABOLIC CONSTANTS FOR CHLOROFORM IN HUMAN MICROSOMES.

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Chloroform ($CHCl_3$) is a member of the trihalomethane family of water disinfection by-products and is classified as a 2B carcinogen by IARC. Cytochrome P450 2E1 is responsible for most of the metabolic clearance of $CHCl_3$, resulting in the formation of phosgene, which is implicated in its toxicity. Because of the role of hepatic bioactivation in the toxic mechanism and the lack of kinetic parameters for $CHCl_3$ metabolism in human samples *in vitro*, this project was undertaken to examine human inter-individual variation in the metabolism of $CHCl_3$. Ten individual samples of microsomal protein (MSP) isolated from liver tissue from adult human organ donors were selected for analysis based on their representativeness of the CYP2E1 contents observed in the general sample (20 - 100 pmol CYP2E1/mg MSP). The vial equilibrium headspace technique was employed for determination of metabolic rate constants (kinetic parameters) for $CHCl_3$. Incubations at 37°C using each of the ten human hepatic MSP preparations individually were conducted at initial $CHCl_3$ concentrations of 0.4, 1.4, 9.4, and 38.6 ppm and the removal of $CHCl_3$ from the headspace was determined by gas-chromatography with electron capture detection. $CHCl_3$ removal demonstrated a time- and protein-dependent loss of $CHCl_3$ from headspace, which was dependent on the addition of cofactor. We observed substantial differences in the metabolism of $CHCl_3$ among samples derived from the ten organ donors, including significant correlation with hepatic 2E1content. In summary, this *in vitro* approach has proven useful in evaluating variance in human metabolism for chloroform and should be explored with other volatile organic compounds. Measures of variability in metabolism may be incorporated in physiologically-based pharmacokinetic (PBPK) models to reduce uncertainties in risk assessment. (This abstract does not reflect EPA policy).

1133 EFFECTS OF PREGNANCY AND DIET ON PULSATILE ETHANOL EXCRETION IN FEMALE RATS FED *VIA* TOTAL ENTERAL NUTRITION.

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We have developed intragastric infusion systems in which ethanol (EtOH)-containing diets are fed as part of a system of total enteral nutrition (TEN). This allows complete control of ethanol and caloric intake. In this model, blood and urine ethanol fail to attain a steady-state, pulsing with a frequency of 6-7 days. Virgin female rats continuously infused 13 g/kg/d EtOH at a caloric intake of 160 $kcal/kg^{3/4}/d$ had mean peak urine EtOH concentrations (UECs) of 369 ± 25 mg/dl and an integrated area under the UEC profile of 2647 ± 164. In contrast, when the caloric intake was increased to 220 $kcal/kg^{3/4}/d$, using the same ethanol dose, the UEC peak was reduced to 200 ± 21 mg/dl ($P \leq 0.05$) and the integrated area reduced by over 50% to 1253 ± 264 ($P < 0.05$). When pregnant female rats were infused EtOH at the same dose at 220 $kcal/kg^{3/4}/d$, the optimal caloric intake to meet the nutritional requirements of pregnancy, peak UEC values were further reduced to 76 ± 8 mg/dl with an area of 309 ± 16 ($P \leq 0.05$ *vs.* 220 fed non–pregnant females). Subsequent experiments where pregnant rats were fed EtOH diets only at night, have demonstrated that a difference of 2 g/kg/d in EtOH intake was required to match UEC and blood EtOH concentrations in pregnant and non-pregnant rats. In pregnant females fed 160 $kcal/kg^{3/4}/d$ the UEC values were 325 ± 53 mg/dl (peak) and 1392 ± 283 (area). These values were significantly higher than those for the pregnant rats fed 220 kcal but lower than those for the virgin rats fed 160 kcal. These data suggest that diet and pregnancy both affect ethanol metabolism and clearance (Supported in part by AA08645 T.M.B. and AA12819 M.J.J.R.).

1134 *IN-VITRO* TO *EX-VIVO* EXTRAPOLATION OF INTRACELLULAR BSP METABOLISM PARAMETERS IN RAT LIVER.

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The primary purpose of a mathematical model of the kinetics of a toxin is to extrapolate to conditions/species where an experiment is impossible or undesirable. If the mathematical model is a detailed PBPK model, it is theoretically possible to obtain most or all parameters using in-vitro methods and a minimal number of whole animals. Here we report a study using both isolated perfused rat livers (the ex-vivo part) and S9 preparations from rat livers (the in-vitro part) to compare the intracellular metabolism of BSP. For the perfused rat livers, eight different combinations of perfusate BSA concentration, recirculating *vs.* one-pass perfusion, and initial BSP concentration were employed to enable estimates of membrane transport, and metabolism for both parent BSP and its glutathione conjugate (BSP-GSH). Extracellular protein binding parameters were taken from the literature. The S9 studies included not only metabolism studies to estimate Vmax and Km, but also protein binding studies to enable the proper extrapolation from S9 conditions to intracellular perfused liver conditions. Perfused liver study metabolism parameters

were estimated to be Vmax = 36.1 µmoles/h/liver and Km = 0.018 µM. The S9 study Vmax for whole liver was ~151 µmoles/h/liver and the apparent Km (before correcting for protein binding in S9) was 7.5 µM. Protein binding of BSP in S9 fit best with 2 binding sites, one site having a Bmax of (3.8 * scale-up factor, s9 to liver) mM and a Kd of 1.7 mM, and the second having a Bmax of (27.5 * scale-up factor) mM and Kd of 230 mM. Applying binding values to the S9 metabolism studies produced an estimated true Km of 0.015 mM (based on estimate of free BSP for total BSP of 7.5 mM and a scale-up factor of 200). This study shows promise for the process of using in-vitro experiments for ex-vivo or in-vivo models, and presents issues that must be considered for proper extrapolation.

1135 13 –WEEK DIETARY AND DEVELOPMENTAL TOXICITY STUDIES OF POLYDIMETHYLSILOXANES (PDMS) IN RATS AND RABBITS.

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Potential hazards of two PDMS fluids, 10 and 350 cSt were evaluated in studies which included 13-week dietary and developmental toxicity studies in rats and rabbits, respectively. In the dietary studies, 15 male and 15 female F-344 rats were fed Purina® certified chow mixed with the respective test articles at concentrations up to 5000 ppm for 13 weeks. In the developmental toxicity studies, the test articles were administered by dermal applications to 3 groups of 23 pregnant female New Zealand white rabbits from Day 6 to 19 after mating at dosages of 33, 300 and 1000 mg/kg/day. Test article administration in the feeding studies produced yellow matting at the anogenital region of males and females in the 5000 ppm group administered 10 cSt fluid, presumably as a result of anal leakage of the test article; increased mean food consumption that may be due the high concentration of the non-nutritive test article in the diet and dose related increases in the corneal opacities that may be associated with a local irritative effect of the test article in the eyes. In the developmental toxicity studies, one female in the control group aborted and one in each of the 33 and 1000 mg/kg/day groups of 10 cSt fluid had total litter resorptions. One female treated with 1000 mg/kg/day 350 cSt fluid aborted and one female in the control group had total litter resorptions. The incidence and distribution of these findings were not indicative of a test article effect. Litter parameters were considered to be unaffected and fetal examination revealed a number of abnormalities in both the control and treatment groups; however, none were considered to be attributable to treatment with PDMS fluids. Supported in part by Silicones Environmental, Health and Safety Council of North America.

1136 MULTIGENERATIONAL 5-AZA-2'-DEOXYCYTIDINE ABNORMAL DEVELOPMENT.

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Genomic imprinting plays a critical role in fetal growth and behavioral development, and it is regulated in part by methylation of cytosine in DNA. 5-aza-2'-deoxycytidine (d-AZA) is a potent inhibitor of DNA methylation thus controlling gene expression. Changes in methylation patterns can lead to heritable alterations in gene expression leading to abnormal growth and/or morphological development. It has been suggested that epigenetic defects in germline cells due to loss of methylation can be repaired by recombination at meiosis but that some are transmitted to offspring. The aim of this study was to determine if the DNA demethylating agent d-AZA would induce transmissible alterations in development. A multigenerational study was designed in which three generations were examined. At gestation day (GD) 10, pregnant mice were administered 1 mg/kg of d-AZA. Male and female treated offspring were mated avoiding consanguinity . In each generation, 50% of pregnant mice were killed and used for fetal necropsy and skeletal analysis while the other 50 % were allowed to give birth to produce the next generation. Leg and tail abnormalities, abnormal male mating behavior and retarded post natal body development, were observed in F1 d-AZA offspring. In the F2 generation, retarded female body development and cleft palate were observed. Also, there was abnormal development of the third ventricle of the brain. Presence of hydronephosis, reduced ossification of caudal vertebrae, supernumerary thoracic ribs, reduced ossification of the sternum and increased number of rib-sternum attachments were statistically different when compared to controls. In the F3 generation, nares malformations, cleft palate, reduced caudal vertebrae ossification, supernumerary thoracic ribs, and increased number of rib-sternum attachments were statistically significant in relation to controls. Our data suggest that altered methylation patterns (induced initially by d-AZA embryonic exposure) may be inherited by the offspring of exposed parents leading to altered imprinting and development

1137 CONTROL VAGINAL OPENING DATA FOR F1 GENERATION CRL SPRAGUE-DAWLEY ("GOLD STANDARD") RATS IN EPA DEVELOPMENTAL NEUROTOXICOLOGY, EPA MULTIGENERATION AND FDA PERI-POSTNATAL STUDIES.

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Vaginal opening and balano-preputial separation are classically considered to be endocrine-mediated developmental endpoints indicative of sexual maturation in the rat that are generally independent of body weight. Because these events do not occur until after postnatal day 21, the usual day rat pups are weaned, the reported values are usually based on observations for 1 male and 1 female weanling rat per litter. Currently, there is considerable controversy regarding the adequacy of using one pup/sex/litter to represent the litter, because it has been shown for body weights and uterine weights that this practice can result in statistically significant differences reflecting random selection. However, historically, one/weanling rat/litter has been evaluated for these parameters in EPA-compliant Developmental Neurotoxicology and Multigeneration Studies, as well as in FDA-compliant Peri-Postnatal Studies. To identify whether evaluating additional animals was not only practical, but also provided improved data sets, we observed three rats/sex/litter in seven Developmental Neurotoxicology Studies and compared these individual and mean/litter data with that obtained in three Multigeneration Studies and seven Peri-Postnatal Studies. The results of these activities indicate: (1) The mean day of vaginal opening ranges from day 31.6 to day 34.0, based on 60 rats/group (3/litter, 7 Developmental Neurotoxicology Studies). (2) When 1 rat/litter (25/group; 7 Peri-Postnatal Studies) is evaluated, the mean day for vaginal opening ranged from 32.4 to 34.0. (3) Individual values varied remarkably among control animals, indicating that statistically significant differences may be associated with random selection of animals (individual values ranged from postnatal day 28 to 41). (4) Use of 3 rats/sex/litter improved the sensitivity of the assay. (5) There appeared to be a correlation between maternal age at GD 0 and age at vaginal membrane separation.

1138 VALIDATION OF THE MORRIS MAZE AS A TEST OF LEARNING AND MEMORY IN YOUNG RATS – SUITABILITY FOR DEVELOPMENTAL TOXICITY STUDIES.

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To assess the suitability of the Morris water maze as a test of learning and memory in young rats, performance of CD rats was assessed at 4 and 9 weeks of age after administration of Scopolamine. Groups of 10 male and 10 female rats were dosed by intraperitoneal injection with Scopolamine hydrobromide at 1.5 mg/kg/day on each of days 28-30 or 63-65 of age. Twenty minutes after each dose the rats were placed individually in the Morris water maze - a circular pool (0.9 m diameter days 28-30; 1.4 m diameter days 63-65) filled to a depth of 30 cm with water at approximately 29 °C made opaque using a non toxic opacifier. A similarly constituted Control group received saline vehicle. A platform was located at a fixed point in the pool, concealed below the surface of the water. Each animal received 3 trials on each of 3 consecutive days of testing. In each trial, the rat was placed in the water at the perimeter of the pool and allowed a maximum of 90 seconds to find the platform. The time to reach the platform and the number of pool sectors crossed were recorded. Treatment of rats with Scopolamine on both Days 28-30 and Days 63-65 of age resulted in a general impairment of performance in the water maze - for both sexes there were some statistically significant increases in mean trial times and mean number of sectors entered compared with the respective Controls. In contrast to Controls, which showed improved performance over the 3 days of testing, performance of treated males and females on Days 28-30 of age only improved between Days 2 and 3 of testing. At Days 63-65 of age, treated males showed improved performance over the 3 days of testing but females showed no improvement. These results demonstrate the suitability of the Morris water maze for assessing learning ability on regulatory developmental toxicity studies.

1139 THE USE OF THE MOUSE FOR EVALUATION OF PRENATAL DEVELOPMENTAL TOXICITY AND REPRODUCTION AND FERTILITY EFECTS.

M. E. Moxon and R. W. Lewis. *HAES, Syngenta CTL, Macclesfield, United Kingdom.* Sponsor: E. Lock.

In regulatory toxicology, the rat is the guideline preferred species for evaluation of prenatal developmental toxicity and for evaluation of reproduction and fertility effects. However, in some instances the rat may not be the most appropriate model i.e. when the rat is not the most relevant species for human health risk assessment. Within CTL, the mouse has been developed successfully as an alternative rodent

species for these evaluations. For evaluation of prenatal developmental toxicity, the technical challenges due to the change of species included control over the time of mating and hence control over the time of caesarean delivery. The purpose of this control was to optimise the size of the foetuses for evaluation and to avoid premature delivery of litters. Other challenges included the methodology for visceral examination of small foetuses by dissection under magnification, the preparation of double-stained skeletons and their subsequent examination. The test system is compliant with current OECD and EPA Guidelines. There were fewer technical challenges involved in using the mouse for evaluation of reproduction and fertility effects. Selection of the strain of mouse was an important factor particularly with respect to mating characteristics, i.e. the observation of a copulation plug needed to be a reliable indicator of successful mating, and litter size and pup survival needed to be adequate for continuation of the study over two generations and meaningful evaluation of the data. Currently, methodology is being developed to investigate oestrus cyclicity, examination of the post lactational ovary and sperm analysis to achieve compliance with the current OECD and EPA Guidelines.

1140 CONTROL PRIMORDIAL FOLLICLE COUNTS IN MULTIGENERATION STUDIES IN CRL SPRAGUE-DAWLEY ("GOLD STANDARD") RATS.

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Ovarian primordial follicle (PF) count is an endpoint in the 1998 EPA multigen. PF counts are identified for ovaries from at least 10 F1 control and high group females, 5 sections/ovary. Means/ovary are determined and compared, lower group values are determined if statistically significant differences are present. Different counts and assay sensitivity are associated with age, number of animals, sections/ovary, section thickness, and criterion defining PF. Generally, 4 PF grades (1, 2, 3a, 3b) are counted (oocyte without surrounding granulosa cells to oocyte ringed by granulosa cells). These criteria were used in 3 multigeneration EPA- and GLP-compliant studies at our laboratories, using the same rat strain, procedures, definitions and evaluator. Variation existed in control PF counts between right and left ovaries of the same rat, mean values for individual ovaries and mean values/group. We have heard that our data differs from that of other groups. In toto, these findings indicate that intra- and inter-laboratory differences exist, and that as currently performed, this endpoint may not be sufficiently reproducible to be considered a pivotal endpoint for human risk assessment. In 2 of our 3 studies, mean and standard deviation control group values were based on 30 rats/group; in the 3rd study, values were based on 10/group. We confirmed age-related differences between P and F1 rats in 1 study (means/ovary: P rats = 9.29 ± 5.32 and 8.89 ± 3.48; F1 rats = 14.50 ± 6.12 and 16.07 ± 5.75). Mean values/ovary for F1 rats in the 3 studies for each ovary = 14.50 ± 6.12 and 16.07 ± 5.75, 13.96 ±7.44 and 13.41 ± 7.49, and 13.64 ± 8.36 and 12.66 ±8.42. Mean PF values/ovary ranged from 2.6 to 44.4. Mean PF of 2.6 = 1, 1, 1, 3 and 7 PF/5 sections; contralateral ovary mean PF = 7.0 (9, 10, 3, 2 and 11 PF/5 sections). Mean PF of 44.4 = 51, 75, 25, 54 and 17 PF/5 sections; the contralateral ovary mean PF = 26 (29, 27, 23, 27 and 24/5 sections). Individual values, criteria and artifact types will be shown.

1141 REPRODUCTIVE EFFECTS OF 1, 2, 3, 4-BUTANE-TETRACARBOXYLIC ACID (BTCA) IN SPRAGUE-DAWLEY RATS WHEN ASSESSED BY THE CONTINUOUS BREEDING PROTOCOL.

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BTCA was evaluated using the multigenerational Continuous Breeding paradigm to reveal potential reproductive toxicity in rats. Beginning on Study Day 1, BTCA was administered daily *via* oral gavage at doses of 0, 100, 250, 500 mg/kg/day to adult male and female rats (N=20). Mating pairs were allowed to produce three litters (F_{1a}, F_{1b}, and F_{1c}). Dosing of the F_1 generation (F_{1c} animals) was initiated on post-natal day (PND) 22. On PND 81±10, F_{1c} (N=20) animals were assigned to mating pairs and allowed to produce three litters (F_{2a}, F_{2b}, and F_{2c}). Endpoints evaluated included body weight, feed consumption, clinical signs, reproductive/litter parameters, sexual development and pathology. No changes were seen in the body weights or feed consumption of the F_0 animals. Decreases of 4-5% were seen in the body weights of the 500 mg/kg F_{1c} males during Week 2, and Weeks 12-16. Feed consumption was decreased during Week 12 for the 100 mg/kg F_{1c} males. Throughout the F_0 and F_1 generations, most reproductive parameters were unchanged in all dose groups compared to the controls with the exception of decreases in the pregnancy indices in the 500 mg/kg pairs compared to control during the second litter of each generation (F_{1b}; 13/17 compared to 20/20, F_{2b}; 16/20 compared to 20/20). Sperm parameters and vaginal cytology were unchanged in the F_0 and F_1 generations. The remaining reproductive changes were not considered to be treatment-related. The only microscopic lesion related to treatment was a renal lesion consisting of the presence of multinucleated giant cells, often centered around acicular crystalline material in both F_0 and, at a decreased incidence, F_1 generations. Excluding several incidental changes, BTCA produced no reproductive toxicity but did produce general toxicity at 250 and 500 mg/kg, as noted by the microscopic renal lesions seen in both the F_0 and F_1 generations. Based on the results of this study, BTCA would not be considered a reproductive toxicant.

1142 ANTIANDROGENIC EFFECTS OF A PHTHALATE COMBINATION ON *IN UTERO* MALE REPRODUCTIVE DEVELOPMENT IN THE SPRAGUE-DAWLEY RAT: ADDITIVITY OF RESPONSE?

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Environmental antiandrogens constitute a class of chemicals that significantly affect reproductive development in laboratory animals. In the environment it is more likely that exposure will occur to multiple agents that may have similar or different mechanisms of antiandrogenicity. The objective of the present study was to examine the reproductive developmental effects on male rats exposed to two phthalates *in utero*, both as individual compounds and in combination. Dose levels were selected based on LOAELs for reproductive development obtained from previous dose-response studies. Pregnant Crl:CD(SD)BR rats, 10 per group, were treated by gavage with either corn oil vehicle, 100 mg/kg/day of di(n-butyl) phthalate (DBP) or di(2-ethylhexyl) phthalate (DEHP) or a combination of each phthalate at 100 mg/kg/day in combination (effectively 200 mg/kg/day of phthalate) on gestation days 12 to 21. On postnatal day (PND) 1 the anogenital distance (AGD) and male pup body weights were measured and each individual pup was uniquely identified with a footpad tattoo. On PND 13 the retained areolae were counted on each male pup. At necropsy on PND 90 the AGD was again measured and the rats were shaved to count permanent nipples. Organ weights were recorded for the testes, epididymides, prostate (dorsolateral and ventral) and seminal vesicles. On PND 1 the AGD was reduced significantly in the combination group while it did not differ from control for DBP or DEHP alone. Significantly increased numbers of areolae were retained on PND 13 in the males in the combination group but not in the other two phthalate groups. There were no consistent changes in organ weights and only a small number of reproductive tract lesions were seen in the phthalate-exposed animals. In line with default assumptions, the effect of prenatal exposure to a combination of two phthalates on AGD and nipple retention in male rats was additive in comparison with the effect of exposure to each phthalate alone.

1143 PERMANENT ALTERATION OF ANOGENITAL DISTANCE AND NIPPLE RETENTION IN MALE RATS EXPOSED TO DI(N-BUTYL) PHTHALATE *IN UTERO*.

N. J. Barlow, B. S. McIntyre and P. M. Foster. *CIIT Centers for Health Research, Research Triangle Park, NC.*

Di(n-butyl) phthalate (DBP) has been shown to act as an antiandrogen when male rat fetuses are exposed during late gestation. Antiandrogenic effects noted include decreased anogenital distance (AGD) on postnatal day (PND) 1 and increased retention of areolae on PND 13. The objective of this study was to compare the AGD and areolae/nipple retention data obtained during the early postnatal period to those obtained in adulthood to determine if the changes were permanent. Pregnant Crl:CD(SD)BR rats, 10 per group, were treated by gavage with either corn oil vehicle or DBP at 100 and 500 mg/kg/day on gestation days (GD) 12 to 21. The AGD and male pup body weights were measured on PND 1 and retained areolae were counted on PND 13. These end points were also collected at necropsy on PND 180. Pups exposed to 500mg/kg/day exhibited a significant, 14%, decrease in AGD on PND 1. At necropsy these DBP-exposed offspring exhibited a significant, 10%, reduction in AGD. The reduction in AGD at PND 1 correlated with the reduction in AGD at PND 180 ($r^2 = 0.80$). Similar effects were seen in areolae/nipple retention with significantly increased numbers of retained areolae/nipples in the high dose group at both PND 13 and 180. Lesions in the male reproductive tract included atrophic testes accompanied by hypoplastic or malformed epididymides, malformed seminal vesicles and small prostates. The incidence of gross lesions in the reproductive tracts of control animals was ~2%, while at 100 and 500 mg/kg/day they were ~9 and 80%, respectively. These data demonstrate that decreased AGD and increased nipple retention following *in utero* DBP exposure are permanent effects with strong correlation between the data from the early postnatal period and adulthood. As the alterations in AGD and areola/nipple retention are permanent, there is support for the argument that these end points are malformations and should be incorporated routinely into standard testing protocols and considered in risk assessment.

1144 LACK OF DEVELOPMENTAL TOXICITY IN SD RATS WITH DI(2-ETHYLHEXYL)TEREPHTHALATE (DEHT, DOTP).

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A developmental toxicity study was conducted in Sprague-Dawley rats with DEHT. Groups of 25 mated females were exposed to 0, 0.3, 0.6, and 1.0% DEHT in the diet beginning on Day 0 of gestation until termination on Day 20. Mean body weight and body weight changes were reduced for the 1.0% group. Net body weight and body weight change were significantly lower for the 1.0% group compared with controls. On Day 20, dams were euthanatized; the uterus was removed and weighed. The numbers of viable fetuses and early and late resorptions were counted. Each fetus was evaluated for external, visceral and skeletal anomalies. No effects on litter size or mean pup weight were observed. There were no differences in the percentages of viable fetuses or early or late resorptions. No visceral or skeletal effects were seen that could be attributed to treatment. The NOAEL for developmental toxicity was 1.0% in the diet. These data complement the information from Gray et al. (2000) which indicate that DEHT does not produce antiandrogenic-like effects, and the data from a 2-generation reproductive toxicity study indicating a lack of reproductive toxicity.

1145 EFFECTS OF *IN UTERO* FENITROTHION EXPOSURE ON ANDROGEN-DEPENDENT MALE RAT REPRODUCTIVE DEVELOPMENT: A DOSE-RESPONSE STUDY.

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Fenitrothion [O, O-dimethyl-O-(4-nitro-m-tolyl) phosphorothioate] is an organophosphate insecticide that is known to antagonize the androgen receptor. The objective of the present study was to evaluate the ability of fenitrothion to disrupt androgen-dependent sexual differentiation in the male rat. Pregnant Crl:CD(SD)BR rats were administered fenitrothion by gavage at 0, 5, 10, 15, 20, or 25 mg/kg/day (n=6-11/group) from gestation day (GD) 12 to 21. Maternal toxicity was observed in the dams treated with 20 and 25 mg fenitrothion/kg/day based on tremors and a significant decrease in body weight gain between GD 12 and 21. Embryo-fetal death was increased in the 20 and 25 mg/kg/day exposure groups as evidenced by a significant decrease in the proportion of pups born alive. *in utero* exposure to fenitrothion altered androgen-dependent sexual differentiation, confirming previous findings that fenitrothion is an androgen receptor antagonist *in vivo.* Anogenital distance (AGD) in the male offspring on postnatal day (PND) 1 was decreased relative to the control group by 8% ($p=0.06$) and 16% ($p<0.001$) in the 20 and 25mg/kg/day dose groups, respectively, with body weight as a covariate. Fenitrothion exposure significantly increased areola retention in male rats on PND 13 from 1.25 ±0.46 in the control group to 4.31 ±0.66 areolae per rat in the 25 mg/kg/day dose group (litter means ± SE). These alterations in AGD and areola retention were transient and not observed at sexual maturity (PND 100). Fenitrothion exposure did not induce a dose-responsive increase in the incidence of gross lesions of the male reproductive tract. Body weights and organ weights were unaltered in the adult male offspring. Fenitrothion exposure induced cholinergic stress in the dams and fetal mortality at the same dose level as that found to elicit the transient but significant effects on AGD and nipple retention in the male offspring.

1146 VALIDATION OF DEVELOPMENTAL NEUROTOXICITY ENDPOINTS IN RATS ADMINISTERED METHIMAZOLE IN DRINKING WATER.

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The purpose of this study was to validate procedures outlined in the USEPA OPPTS 870.6300 guideline for developmental neurotoxicity (DNT) studies. Pregnant Sprague-Dawley rats (N=25/group) were administered either 0, 10, 30 or 100 ppm methimazole (used to treat human hyperthyroidism) in drinking water from gestation day 6 through postnatal day (PND) 21. Endpoints evaluated in the dams included a modified functional observational battery (mFOB), body weights, and food and water consumption. In the pups, brain weights, qualitative neuropathology and quantitative brain morphometry were evaluated at PND 11, 22 and 72. Auditory startle, locomotor activity, learning and memory (Biel Maze) and mFOB were assessed in the offspring repeatedly throughout their development. During lactation, the dams in all three exposure groups weighed significantly less, and consumed less food and water than controls. Locomotor activity was reduced on PND 13 and increased on PND 21 for 30 and 100 ppm offspring relative to controls. Auditory startle response endpoints (Vmax, Tmax and Vave) were affected in the 30 and 100 ppm offspring at PND 20 and 60. Pup body weights were reduced from PND 1-70 for both sexes in all exposure groups relative to controls. Delayed sexual maturation (balanopreputial separation and vaginal patency) was readily apparent in all three exposure groups, except for vaginal patency at 10 ppm. In addition, on PND 11 and 22 both male and female offspring mean brain weights (100 ppm) were reduced relative to controls. Brain weights were reduced on PND 72 for 100 ppm male offspring only. No dam or offspring exposure-related deficits were noted in the mFOB at any time point. Qualitative neuropathology and quantitative morphometry at PND 11 (comparing immersion and perfusion fixation at PND 11), 22 and 72, along with learning and memory data, will be presented. The data analyzed thus far are being used to assess the appropriateness of methimazole as a positive control agent for DNT studies.

1147 REPRODUCTIVE EFFECTS OF DIETHYLHEXYLPHTHALATE (DEHP) IN SPRAGUE-DAWLEY RATS WHEN ASSESSED BY THE CONTINUOUS BREEDING PROTOCOL.

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DEHP was evaluated using a multi-generational protocol in rats to assess potential reproductive effects over multiple generations. Beginning on Study Day 1, DEHP was administered *via* the diet at doses of 1.5 (control), 10, 30, 100, 300, 1000, 7500, and 10, 000 ppm to adult male and female rats (N=17). Each mating generation was allowed to produce three litters (F_{1a}, $_{1b}$, $_{1c}$, F_{2a}, $_{2b}$, $_{2c}$, F_{3a}, $_{3b}$, $_{3c}$). On PND 81 ± 10, animals from the third litter from each generation were assigned to mating pairs (N=17). Reductions were noted in mean body weight of animals receiving 7500 or 10, 000 ppm. Reproductive/litter effects including decreases in pregnancy indices for most generations; decreases in the no. of males per litter and the total no. of pups per litter; decreases in male and female pup weights at birth and during lactation; decreases in anogenital distance for male pups; and delay of sexual development parameters were noted at the top 2 dose groups. The 10, 000 ppm animals did not produce any F_2 generation animals. Outbreedings at 7500 and 10, 000 ppm of treated males with naive females revealed decreases in mating, pregnancy and fertility indices and decreases in the number of implantation sites. Sperm endpoints were decreased at 7500 ppm in all generations and at 10, 000 ppm in the F_0 and F_1 generations. Organ weight changes of the liver (increase), kidney (increase), and male accessory sex organs (decrease) occurred at 1, 000, 7500, and 10, 000 ppm. Treatment-related microscopic findings were noted at 7500 and 10, 000 ppm in the testes, epididymides, liver, adrenal, and kidney in all generations and in the liver at 1, 000 ppm in the F_1 and F_2 generations. DEHP is considered a reproductive toxicant at 7500 and 10, 000 ppm. There was no general or reproductive effects observed at dose levels at or below 1, 000 ppm except liver toxicity at 1, 000 ppm.

1148 DECREASE IN THE LITTER SIZE OBSERVED IN FEMALE RATS INTRAVENOUSLY GIVEN L-CYSTEINE.

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There are few reports on the reproductive and developmental toxicity of amino acids. We previously observed marked decrease in the litter size in female rats intravenously received a high dose of L-Cysteine (CySH) before mating. In the present study, we investigated the oocytes morphologically before and after mating to clarify the cause of the decrease in the litter size observed in the rats. SD rats, 11 weeks old, were intravenously infused CySH at a dose of 1000 mg/kg for more than 1 week before mating. The unfertilized oocytes (UO) collected from ampulla of uterine tubes and fertilized embryos (gestation day [GD] 1 and 2) collected from oviducts were examined morphologically by stereomicroscope. Morfometry was also performed for UO. The number of females in the CySH group from which UO could be collected was the same with that in the control group (non-treatment group), but the number of observed oocytes was a half of that in the control group. Moreover, zona pellucida (ZP)-lacking or partially ZP-lacking oocytes were observed in the CySH group. The morfometry of UO in the CySH showed similar diameter as compared with that in the control group, but ZP became thin. The number of female rats from which embryos were collected on GD 1 in the CySH group was similar to that in the control group, but the number collected on GD 2 was reduced to a half of that in the control group. The number of observed embryos in dams on GD 1 and GD 2 were decreased markedly in the CySH group, and most of them were degenerative embryos. It was observed in this study that a high dose of CySH induced the lacking or partially lacking of ZP or thinning of ZP in UO and embryos on GD 1 and GD 2. It is known that ZP plays an important role in

early embryogenesis. The oocytes, which were affected at ZP by CySH treatment, were degenerated. In conclusion, affected ZP was considered to contribute to the decrease in litter size.

1149 CONTROL PREPUTIAL SEPARATION DATA FOR F1 GENERATION CRL SPRAGUE-DAWLEY ("GOLD STANDARD") RATS IN EPA DEVELOPMENTAL NEUROTOXICOLGY, EPA MULTIGENERATION AND FDA PERI-POSTNATAL STUDIES.

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Balano-preputial separation and vaginal opening are classically considered to be endocrine-mediated developmental endpoints indicative of sexual maturation in the rat that are generally independent of body weight. Because these endpoints do not occur until after postnatal day 21, the usual day rat pups are weaned, the values are generally based on observations for one male and one female weanling rat/litter. Currently, there is considerable controversy regarding the adequacy of using one weanling rat/sex/litter to represent the litter, because it has been shown, for body weight and uterine weights, that this practice can result in statistically significant differences reflecting random selection. However, historically, one rat/litter has been the number evaluated for these parameters in EPA-compliant Developmental Neurotoxicology and Multigeneration Studies, as well as in FDA-compliant Peri-Postnatal Studies. To identify whether evaluating additional animals was not only practical, but also provided improved data sets, we evaluated 3 rats/sex/litter in 7 Developmental Neurotoxicology Studies and compared these individual and mean/litter data with that obtained in 3 Multigeneration Studies and 7 Peri-Postnatal Studies. The results of these activities indicate: (1) The mean day of preputial separation ranged from days 45.2 to 48.4, based on 60 rats/group (3/litter, 7 Developmental Neurotoxicology Studies). (2) When 1 rat/litter (25/group; 7 Peri-Postnatal Studies) was evaluated, the mean day for vaginal opening ranged from 44.6 to 48.1. (3) Individual values varied remarkably among control animals, indicating that significant differences observed between studies may be associated with random selection of animals to be evaluated (individual values ranged from postnatal days 40 to 76). (4) Use of 3 rats/sex/litter improved the sensitivity of the assay. (5) There appeared to be a correlation between the age at sexual maturation and maternal age at GD 0.

1150 ROLES OF PROGESTERONE ON SUPPRESSION OF UTERINE DECIDUALIZATION AND IMPLANTATION FAILURE INDUCED BY DIPHENYLTIN DICHLORIDE (DPTCL) IN RATS.

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In our previous study, administration of DPTCl at 16.5 mg/kg and higher on days 0-3 of pregnancy was found to induce implantation failure in rats (Toxicol. Lett., 108, 17-25, 1999). In the present study, the effects of DPTCl on uterine function, as a cause of implantation failure, and the roles of progesterone in DPTCl-induced implantation failure were determined in rats. Female rats were given DPTCl by gastric intubation at 4.1, 8.3, 16.5, or 24.8 mg/kg on days 0-3 of pseudopregnancy and the decidual cell response was induced on day 4 of pseudopregnancy. The uterine weight on day 9 of pseudopregnancy served as an index of the uterine decidualization. A significant decrease in the uterine weight, which indicates suppression of the uterine decidualization, was detected at 16.5 and 24.8 mg/kg. The ovarian weight and number of corpora lutea in the DPTCl-treated groups were comparable to the controls. A significant decrease in the serum progesterone levels was found at 16.5 and 24.8 mg/kg. Hormonal regimen supported the decidual development in ovariectomized rats given DPTCl. The pregnancy rate and number of implantations in the groups given DPTCl at 16.5 and 24.8 mg/kg on days 0-3 of pregnancy in combination with progesterone on days 0-8 of pregnancy were higher than those in the groups given DPTCl alone. It could be concluded that implantation failure due to DPTCl is mediated *via* the suppression of uterine decidualization correlated with reductions in serum progesterone levels.

1151 HOMOLOGOUS RECOMBINATION AS A POTENTIAL MECHANISM FOR PHENYTOIN-INDUCED TERATOGENICITY.

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Epilepsy treatment involves the use of anticonvulsants such as phenytoin (**PHY**). PHY is teratogenic in numerous species, but the mechanism by which it initiates teratogenicity remains to be elucidated. PHY can be enzymatically bioactivated to a reactive intermediate capable of damaging essential macromolecules including DNA. PHY can induce DNA double-strand breaks (**DSB**), which may be repaired through homologous recombination (**HR**). Increased DSB levels may induce hyper-recombination that can lead to deleterious genetic changes. We hypothesize that these changes, such as gene deletions, may be an important molecular mechanism for chemical-induced teratogenesis. We used a Chinese hamster ovary (**CHO**) cell line with a *neo* direct repeat recombination substrate (**CHO 3-6**). The 5' *neo* is driven by the dexamethasone (**DEX**)-inducible mouse mammary tumor virus promoter; the second *neo* lacks a promoter. HR frequencies were determined by counting the number of G418-resistant colonies per live cell, and recombinant products were analyzed by Southern blot to distinguish gene conversions without an associated crossover from deletions, which can occur by crossing over or single-strand annealing. CHO 3-6 cells were treated with PHY at 80, 240 and 800 μM for 4 or 24 hr, and the 24 hr experiments were performed in the presence or absence of DEX. We found that PHY enhances HR in a dose-dependent manner, and that PHY-induced HR is further enhanced by increased transcription of the 5' copy of *neo*. Of more than 10 HR products analyzed to date, all arose by gene conversion without associated crossover. These results are similar to HR stimulated by I-*Sce*I nuclease-induced DSBs, but contrast with the high rate of deletions seen with spontaneous and UV-induced HR. Thus, our data suggest that PHY-initiated DNA damage induced HR proceeds through a DSB intermediate, implicating HR as a potential mechanism for chemical-induced teratogenesis.

1152 STUDIES OF EMBRYOTOXIC EFFECTS OF BITUMEN-SALT MASSES (BSM) AND THEIR AQUEOUS EXTRACTS CONTAINING GB, GD, OR VX TWO-STAGE DESTRUCTION PRODUCTS.

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In support of Cooperative Threat Reduction activities, the Research Institute of Hygiene, Occupational Pathology and Human Ecology in St. Petersburg, Russia participated in joint studies with the U. S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) to investigate the potential reproductive effects of materials leached from the bituminous-salt mass (BSM) end-products of the Russian two-stage chemical agent destruction process. These studies were conducted to address concerns that materials leached from the BSMs could pose a health threat to receptors in the vicinity of future storage/disposal sites. Using aqueous leachates or oil extracts derived from Russian bitumen alone, or BSM containing demilitarized Russian manufactured sarin (GB), soman (GD) or VX, embryotoxicity was evaluated *in vitro* using pre- and post-implantation rodent embryos cultured in blood sera. Embryos were exposed either directly to aqueous extracts or indirectly by using serum obtained from donor animals that were exposed orally to oil suspensions of bitumen or the various BSMs for different durations. Developmental anomalies included dismorphogenesis, decreased cleavage rates, yolk sac edema, hemorrhages (pericardial sac, brain, allantois base) and decreased brain dimensions. Diluting extracts to 4% eliminated their embryotoxic action. In other studies, embryo/fetolethal effects were seen in embryos of pregnant rats orally exposed to aqueous extracts of bitumen, GB-, GD-, or VX-BSM (4, 20 or 100%) daily during gestational days 1-19. Embryos showed the highest sensitivity to BSM-VX aqueous extracts. In summary, BSM aqueous extracts possess weak embryotoxic properties that decrease proportional to decreasing concentrations. While all BSMs demonstrate a measure of embryotoxicity, BSM-VX is the most toxic for embryonic development.

1153 THE EFFECTS OF CONTINUOUS INTRAVENOUS SALINE INFUSION UPON CLINICAL PATHOLOGY PARAMETERS IN THE WEANLING ALBINO RAT.

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The use of pharmaceuticals and biotechnology products to treat pediatrics requires that juvenile animal studies be considered prior to conducting clinical trials. The weanling rat represents a suitable model for testing infusion products since it allows for the use of the clinical dose route in the pre-clinical testing. In this study, animals were cannulated at Day 22 post partum using routine surgical procedures and thereafter infused continuously with physiological saline at rates up to 5 mL/kg/h up to 43 days post partum. Terminal blood sample were collected on Days 37 and 44 post partum from the abdominal aorta and analyzed for a standard panel of hematology and clinical biochemistry parameters. There were only minor effects upon clinical condition and a general lack of, or minimal, local tissue damage or inflammatory reaction seen pathologically at the infusion site in infused animals. Consequently, the clinical pathology data generated for these animals allowed for a direct evaluation of the potential changes associated with saline infusion. Although a slight reduction in white blood cell numbers was observed in infused animals *versus* age-matched historical controls, the values recorded for the infused weanlings

remained within our laboratory reference ranges for older (8 to 12 week old) rats. The differences between the remaining clinical pathology parameters of infused *versus* non-infused animals were minor in nature and likely represented biological variation. Consequently, it was concluded that continuous intravenous saline infusion did not adversely affect the clinical pathology of weanling rats thus confirming the suitability of this animal model for testing infusion products.

1154 CONTINUOUS INTRAVENOUS INFUSION IN THE WEANLING ALBINO RAT.

S. N. Groom, K. Robinson, G. Washer, H. Perras and M. Guimont. *Toxicology, CTBR, Senneville, PQ, Canada.* Sponsor: G. Lulham.

The use of pharmaceuticals and biotechnology products to treat pediatrics requires that juvenile animal studies be considered prior to clinical trials. The weanling rat represents a suitable model for infusion products since it allows for the use of the clinical dose route in the pre-clinical pediatric testing. For over a decade, our laboratory has conducted developmental/reproductive studies in rats and rabbits using the infusion route and recently applied our experience with infusion technology to weanling rats. Using aseptic techniques, 40 weanling rats were anesthetized and surgically implanted *via* the femoral vein with a medical-grade silicone-based catheter at 22 days of age. Animals were fitted with a jacket and tether infusion apparatus that was suitably scaled for their size and were infused with physiological saline *via* an in-line 0.µm filter, for 24 hours/day, up to 42 days of age. Dosimetry data for the infusion rates employed (2.5 and 5.0 mL/kg/h) confirmed animals received within ±10% of the nominal dose volume at each infusion rate over the course of the investigation. There were only minor effects upon clinical condition, body weight and clinical pathology parameters and these data are presented. The general lack of, or minimal, local tissue damage or inflammatory reaction seen pathologically at the infusion site suggests that the infusion interval could be extended beyond 42 days of age, if required. In conclusion, weanling rat infusion provides an appropriate animal model, to assess the safety and pharmacokinetics of infused drug products intended for pediatric use prior to initiation of clinical trials in human pediatric populations.

1155 AN ASSESSMENT OF THE USE OF DIMETHYL SULFOXIDE AS A SOLVENT FOR HYDROPHOBIC CHEMICALS IN A DROSOPHILA-BASED DEVELOPMENTAL TOXICITY PRESCREEN.

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Dimethyl sulfoxide (DMSO) is routinely used in toxicity testing to solubilize hydrophobic chemicals; however, the potential of DMSO to induce developmental toxicity in the Drosophila-based prescreen has not been well characterized. The purpose of this study was to investigate this toxicity and to determine a maximum concentration of DMSO which can be used. Three separate experiments were conducted with DMSO (CAS# 67-68-5; Sigma D-5879, 99.5%) at concentrations ranging from 0.05 - 150 µl/vial. Drosophila were exposed throughout development (egg through third instar larva) in culture vials to medium containing DMSO. A mated, untreated, Oregon-R wild-type female was added to each vial and allowed to oviposit for 20 hours, then removed. Emerging offspring were collected over 10 days, and examined microscopically (25x) for bent humeral bristles and wing blade notches; morphological defects shown to occur with an increased incidence in flies exposed to developmental toxicants. In each experiment, the incidence of the two defects at each concentration was compared to the concurrent controls using chi-square. Where replicate data were available at a given concentration, incidence data were also pooled and compared to the pooled controls. No flies emerged at concentrations greater than 50 µg/vial. In the first experiment the incidence of bent humeral bristles was significantly increased at 25 µl/vial, 64/246 ($p < 0.001$) and at 50 µl/vial, 2/2 ($p < 0.001$); in the second experiment bristle defects were increased only at 50 µl/vial, 5/8 ($p < 0.001$); in the third experiment bristle defects were increased at 1.0 µl/vial, 5/155 and 5.0 µl/vial 5/146 (both $p < 0.05$). These results indicate that higher concentrations of DMSO can increase the incidence of bristle defects in developing flies. However, based on the lack of a statistically significant increase in defects at lower concentrations in the pooled data across 3 experiments, the data suggest that DMSO at a concentration of 10 µl/vial can be used to evaluate hydrophobic chemicals in this assay.

1156 MODIFIED DAVIDSON'S FIXATIVE AS A REPLACEMENT FOR BOUIN'S SOLUTION USED FOR FETAL EVALUATION UTILIZING WILSON'S TECHNIQUE.

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Near-term rat fetuses have been traditionally placed in Bouin's solution for fixation and decalcification. This allowed an adequate soft tissue evaluation following razor blade sectioning. While Bouin's solution produced good results, the contrast of the tissues could be improved. The picric acid component presents a health and safety hazard, and an environmental disposal problem. Davidson's fixative was modified at Huntingdon Life Sciences by H. Jonassen to be used as a replacement for Bouin's solution that is routinely used to fix reproductive organs, testes and epididymides. The modified Davidson fixative contains 14% of pure ethanol, 6.25% glacial acetic acid, 37.5% saturated formaldehyde and 42.25% distilled water. The fetuses are placed in a plastic jar containing modified Davidson's fixative for one week. After fixation, the fetuses are rinsed twice in tap water and stored in 70% Isopropyl Alcohol. The razor blade serial section technique described by Wilson is used to perform visceral evaluation. Superior contrast and enhanced definition of organs and vessels are achieved in the fetuses following fixation in Modified Davidson's fixative. Sections remained moist for an extended period of time during evaluation.

1157 INDUCTION OF STRESS PROTEINS BY ARSENIC COMPARED TO HYPERTHERMIA IN TISSUES OF THE AVIAN EMBRYO.

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Exposure of the early embryo to environmental insults may result in different developmental outcomes depending on target dose, developmental stage, and tissue specificity. The induction of stress proteins may be important indicators of exposure and may influence developmental toxicity. The present study was designed to evaluate the early avian embryo as a model system to study the induction of stress proteins by arsenic and hyperthermia in target tissues. The study compared the induction of the heat shock proteins Hsp24, Hsp70, as well as c-jun and its phosporylated form (P-c-jun) in the head and heart, which differ in stress resistance. K-strain chick embryos at 3 days of development were exposed to graded dosages of sodium arsenite (AS) or to heat shock (HS) of comparable embryo toxicity. Head and heart were harvested at 3, 6, and 10 hr post exposure to AS or HS. Then, stress protein induction was analyzed using immunoblotting analysis. Constitutive levels of Hsp70 and c-jun were similar in the 2 tissues, but constitutive P-c-jun expression was 7-fold higher and Hsp24 was 3-fold higher in the heart. Embryos treated with AS doses of 125, 200, and 250 ug/g demonstrated a dose-dependent increase in Hsp24, Hsp70, and P-c-jun by 10 hr with consistently and substantially higher expression in the heart. C-jun expression in response to AS was similar in the head and heart. AS compared to HS treatments were also evaluated for their capacity to induce stress responses. In the head, HS induced P-c-jun and Hsp24 at a 50-fold differential over comparable AS doses at 3 hr. Hsp70 induction by HS was also greater than AS by about 4-fold. Non-lethal HS treatments were effective at inducing Hsp24 and Hsp70, whereas comparable AS treatments failed to elicit significant responses in head and heart. Thus, profiles of differentially induced stress proteins may be useful as indicators of agent-specific exposures at the tissue level. Also, differential stress protein induction in the developing head and heart may contribute to regulating tissue sensitivity and overall developmental toxicity.

1158 POSTPARTUM DEVELOPMENTAL REFERENCE DATA FROM BEAGLE DOGS OVER FOUR DEFINED PEDIATRIC STAGES.

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The FDA Modernization Act of 1997 and the final FDA rule on April 1, 2001 require companies to address the safety and effectiveness of drugs and biologicals in pediatric patients. One way to address the presumed higher risk in children is to conduct safety evaluations during critical developmental stages using non-rodent animal models. However there is limited reference data available for non-rodent species. This study presents reference data for male and female Beagle dogs for defined developmental stages: term neonate (<3 weeks), infant/toddler (3-6 weeks), child (6-20 weeks) and adolescent (20-28 weeks). These data are derived from 7 to 10 Beagle litters whelped at WIL Research Laboratories, Inc.. The Beagle pups received no treatment over the course of gestational/postpartum development. Mean values and standard deviations are presented for endpoints collected over the course of the study. Parameters evaluated include growth patterns (body weight, height, length and torso circumference); developmental landmarks (incisor eruption, eye lid separation, balanopreputial separation, vaginal patency and testes descent); total body composition (lean vs non-lean tissue) and bone mineral endpoints (bone mineral density and bone mineral content); locomotor activity assessments; and standard clinical chemistry parameters (serum chemistry and hematology) are all presented to demonstrate patterns of change over the duration of Beagle development. The results of this study indicate that over the course of development predictive patterns of physiological changes can be measured and that these patterns can be associated with the defined pediatric developmental stages. Reference data of this kind will be essential for the design of non-rodent pediatric studies and in the in-

terpretation of findings for addressing the potential of pediatric risks from direct product exposure in regulatory toxicology studies employing non-rodent animal models.

1159 AN EVALUATION OF THE POTENTIAL OF TWO PEPTIDES, PRAMLINTIDE AND AC2993 (SYNTHETIC EXENDIN-4), TO CROSS THE HUMAN PLACENTAL BARRIER USING AN *EX VIVO* PERFUSION SYSTEM.

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Pramlintide (a 37-amino acid peptide) and AC2993 (synthetic exendin-4; a 39-amino acid peptide) are under development for treating patients with diabetes mellitus. Because of the chronic nature of diabetes and, thus its treatment, some patients will become pregnant while on therapy. Therefore, it is important to have knowledge of the potential for direct exposure of the developing fetus to these exogenous peptides. The use of human placentas obtained from full-term pregnancies in an *ex vivo* system allows this situation to be studied without the risk associated with an *in vivo* evaluation. The placental cotyledon selected for each perfusion (6 per peptide) met the acceptance criteria set by Challier[1]. Insulin (added as a control peptide known not to cross the placental barrier) and the study peptide were added to the maternal side of the perfusate and both the maternal and fetal sides were sampled over the 2-hour equilibrium perfusion period and assayed for insulin and the study peptide. Measured insulin on the maternal side of the perfusate remained elevated (~4, 000 µU/mL) while the concentration on the fetal remained low (~10 µU/mL). With mean pramlintide concentrations on the maternal side of 15, 000 to 43, 000 pg/mL, the concentrations on the fetal side ranged from 28 to 268 pg/mL (fetal-to-maternal ratio of 0.002 to 0.006). With mean AC2993 concentrations on the maternal side of 1, 200 to 2, 400 pg/mL, the concentrations on the fetal side ranged from 18 to 34 pg/mL (fetal-to-maternal ratio of 0.008 to 0.017). These results suggest that the potential for either pramlintide or AC2993 to cross the human placental barrier is very low. [1] Challier JC. Criteria for evaluating perfusion experiments and presentation of results. Contr. Gynec. Obstet. 1985; 13:32-9.

1160 INCIDENCE OF VARIATIONS AND MALFORMATIONS DURING FETAL DEVELOPMENT IN THE CYNOMOLGUS MONKEY.

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The cynomolgus monkey (Macaca fascicularis) is the predominant species for investigation of embryo-fetal development in the non-human. Given the current momentum of development of new biotechnology products and immunotherapeutics, it is obvious that the demand for developmental toxicity studies in this primate model will increase. In order to be able to distinguish between spontaneous and compound-induced developmental alterations, a comprehensive and reliable data base is mandatory. We therefore investigated 399 fetuses obtained at gestational day 100 and 63 fetuses obtained at gestational day 150 from control mothers. The parameter chosen for assessment was skeletal development. The ossified skeleton was stained with Alizarin red and the carcass was made translucent by preservation in glycerine. A highly graded evaluation scale for identifying even minimal variations was used. At day 100 minor variations were observed in nearly 90% of the fetuses, whereas malformations were encountered in seven fetuses only (1.7%). The most frequent variation was observed in the sternum followed by ribs, vertebral column and skull bones. At day 150 minor variations were also seen in the sternum and ribs at an incidence of 75%. However, malformations could not be detected. These data provide an exellent basis for the characterization of normal fetal development during developmental toxicity studies in the cynomolgus monkey model.

1161 EVALUATION OF TRICHLOROETHYLENE IN A RAT INHALATION DEVELOPMENTAL TOXICITY STUDY.

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As part of a voluntary program to fill "priority data needs" identified by the US Agency for Toxic Substances and Disease Registry (ATSDR), an inhalation developmental toxicity study conforming to current global regulatory guidelines was conducted. Groups of twenty-seven time-mated, female CD rats were whole-body exposed to target concentrations of 0, 50, 150, and 600 ppm vaporized trichloroethylene (TCE) for six hours/day, seven days/week on days 6-20 of gestation in order to evaluate the maternal and developmental toxicity potential of this compound. The level of 600 ppm represents a limit dose defined in EPA guidelines for inhalation studies. In-life parameters evaluated for all groups included clinical observations, body weight, body weight gain, and feed consumption. On day 21 of gestation, all rats were euthanized and examined for gross pathologic alterations. Liver, kidneys, and gravid uterine weights were recorded, along with the number of corpora lutea, implantations, resorptions, and live/dead fetuses. All fetuses were weighed, sexed, and examined for external alterations. Visceral or skeletal examinations, each involving approximately one half of the fetuses, were conducted as designated. Visceral examinations included visualization of the internal structures of the heart. In addition, the internal structures of the head were examined by serial sectioning for one half of the fetuses. Dams exposed to 600 ppm TCE exhibited maternal toxicity as evidenced by significant decreases in body weight gain (22% less than the control group) on gestation days 6 through 9. There were no maternal effects at either 50 ppm or 150 ppm TCE and no indications of developmental toxicity (including teratogenicity) at any exposure level. Therefore, the no-observed-effect-concentration (NOEC) for maternal toxicity was 150 ppm, whereas the embryonal/fetal NOEC was 600 ppm. Funded by the Halogenated Solvents Industry Alliance, Inc..

1162 THE AFFECT OF FUMONINSIN B1 ON FOLATE TRANSPORT IN CELLS DERIVED FROM MURINE EMBRYOS THAT ARE SUSCEPTIBLE TO FB1-INDUCED NEURAL TUBE DEFECTS.

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Fumonisins are mycotoxins that are produced by *Fusarium moniliforme* (*F. verticillioides*) and are contaminants of grains, particularly corn. Their distribution is worldwide and there are at least 14 known fumonisins, of which fumonisin B1 is the most common. FB1 is associated with fatal toxicosis in horses and pigs and it causes hepatotoxicity in rodents. Several studies have linked FB1 to esophageal cancer and neural tube defects (NTDs) in humans. Of the few studies that have investigated the teratogenicity of FB1, most have found very little evidence that this mycotoxin causes any abnormalities other than a generalized embryotoxicity. However, recently we have demonstrated a strain difference to FB1-induced NTDs in two strains of mice, where the pregnant dams were acutely treated during the early stages of gestation. It has been demonstrated that FB1 can disrupt the transportation of folate into cultured cells. Additionally, folate deficiencies have been associated with NTDs in both humans and rodents. Therefore the current study was designed to determine whether FB1 could alter the transportation of folate into cultured primary embryonic cells derived from both susceptible and resistant strains of mice. Once established, primary cells were cultured for 2 days in folate free media during which time a group of cells were also exposed to 20 µg/ml of FB1. At the end of this culture period folate uptake was assayed, using [^{3}H]-folate, from 15 to 60 minutes. Preliminary results from these studies demonstrate that FB1 exposure lead to a decrease in folate uptake in the cells from the susceptible strain. In contrast, the cells from the resistant strain were unaffected by FB1. These differences in folate uptake may lead to a folate deficiency in susceptible embryos and may explain the teratogenic differences seen between these two strains of mice.

1163 *EX VIVO* HUMAN PLACENTAL PERFUSION SYSTEM IN THE ASSESSMENT OF TRANSPLACENTAL PASSAGE.

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Ethical considerations limit *in vivo* human studies of transplacental passage of drugs. Animal testing gives valuable information but the structure and functions of the placenta are species specific. Our aim is to clarify the usefulness of placental perfusion method as a part of pre-clinical risk assessment. Single human placental cotyledons were perfused for 2 hours. Modified Krebs-Ringer phosphate buffer with natriumbicarbonate, dextran and heparin was used as perfusion medium. In successful perfusions the leak from maternal to fetal side was less than 2 ml/h. Carbamazepine (5-carbamoyl-5H-dibenz-[b, f]azepine; CBZ), oxcarbazepine(10, 11-dihydro-10-oxo-5H-dibenz(b, f)-azepine-5-carboxamide; OCBZ) and lamotrigine (3, 5-diamino-6-[2, 3-dichlorophenyl]-1, 2, 4-triazine; LTG) were studied. Antipyrine, a non-ionized, non-metabolized and non-protein bound drug, was used as a reference substance. Drug concentrations were analyzed using high performance liquid chromatography. In addition, cord blood and maternal venous samples were collected from mothers using OCBZ, CBZ and LTG during pregnancy. All studied substrates crossed placenta rapidly, maternal and venous concentrations being equal at steady state. Equilibrium between maternal and fetal concentrations was reached earlier with CBZ, OCBZ and LTG than with antipyrine.

Although data achieved with placental perfusion method usually correlated well with available clinical data differences between *in vivo* and *ex vivo* situation were also noted. Perfusions indicated slight accumulation of 10-OH-CBZ in placental tissue but *in vivo* results indicated no accumulation. This may have been due to tissue oedema during perfusions. In conclusion, human placental perfusion system is a promising method but further studies are needed to validate the method for the assessment of fetal exposure.

1164 INTERLABORATORY STUDY OF THE PRIMARY ANTIBODY RESPONSE TO SHEEP RED BLOOD CELLS IN OUTBRED RODENTS.

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EPA guidelines currently provide a choice in evaluating immune system function in rats and mice using sheep red blood cells (sRBC) as the preferred antigen, namely, an antibody-forming cell (AFC) assay or a sRBC-specific serum IgM ELISA. The initial objectives of this interlaboratory study were for all four laboratories to use the same source of sRBC and determine 1) optimal sRBC concentration for immunization and 2) peak day of antibody response in female CD rats and CD1 mice. The primary objective was for each Lab. to determine which of the two methods was more sensitive in detecting suppression of the antibody response by cyclophosphamide (CP). Each Lab. determined optimal immunization concentrations for each assay and each species. Peak antibody response was day 4 for the AFC in mice or rats, whereas Day 5 was the peak day for the ELISA assay in mice and Day 6 for rats (sRBC injection was on Day 0). Rats were injected iv with sRBC followed by ip injections of 0, 0.3, 1, 3, 10 or 30 mg/kg/day CP. Statistically significant reductions in antibody response were first detected at 1 mg/kg/day (44% suppression, AFC/million splenocytes) and at 10 mg/kg (30-69% suppression by ELISA by 3 labs). Mice were injected iv with sRBC followed by ip injections of 0, 5, 10, 20, 40, or 60 mg/kg/day CP. Statistically significant reductions in the antibody response were detected at 5 mg/kg (36-64% suppression - AFC assay) by 3 labs and 10 mg/kg (55% suppression) by the remaining Lab.. Three labs detected suppression at 10 mg/kg (6-25% inhibition) using the ELISA. In conclusion, the rat ELISA detected significant suppression at the same (2 labs) or lower (1 Lab.) concentration compared to the PFC assay. In contrast, the mouse PFC assay detected suppression at a lower dose than the ELISA in 3 of 4 labs.

1165 THE RESPONSE OF C57Bl/6 MICE TO THE MOUSE INFLUENZA HOST RESISTANCE (MIHR) MODEL.

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In safety testing of potential immune modulators, one of the major objectives is to determine if the compound has an immunosuppressive effect, resulting in an increased susceptibility to infectious disease. Since clearance of the influenza virus requires an intact functional immune system, incorporating cytokine production, antibody production and natural killer cell, macrophage, and cytotoxic T lymphocyte activity, the MIHR model allows an assessment of overall immunocompetence. Although the MIHR model is well characterized in BALB/c mice, there has been limited experience with this model in C57Bl/6 mice. The purpose of this study was to characterize the response of the C57Bl/6 mouse strain in the MIHR model, in order to establish baseline parameters that could be used to assess immune competence in specific knock-out mice on the C57Bl/6 background. Male and female C57Bl/6 mice, 6 - 8 weeks old were administered influenza virus (InV) intranasally [4 X 10^3 (lo InV) or 4 X 10^4 (hi InV) infectious particles], with or without daily oral dexamethasone (DEX) (Day -3 through Day 6 or Day 21) as a positive control. Viral burden in the lung homogenate and InV-specific IgG titers were determined at several time points postinfection. The 10 day survival rate in the C57BL/6 hi InV was 56.6% in males with 10 mg/kg/d DEX, 96.7% in male vehicle controls, 73.3% in females receiving 10 mg/kg/d DEX, and 96.7% in female vehicle controls. In a second study with female mice given 5 mg/kg/d DEX, there were 72% survivors in the hi InV group and 96% survivors in the lo InV group. With 1 mg/kg/d DEX, with hi InV, there were 96% survivors; vehicle control survival was 92-100%. Historical control BALB/c female mice receiving 20 mg/kg/day DEX or vehicle, had 100% 10 day survival. This study demonstrates in comparison to BALB/c mice, the C57Bl/6 strain is more susceptible to InV and DEX in the MIHR, and reinforces that establishing strain-specific characterization of the MIHR is critical for accurate assessment of an immunotoxic potential.

1166 COMPARITIVE ANALYSIS OF HUMAN, CYNOMOLGUS MONKEY AND COMMON MARMOSET PERIPHERAL BLOOD MONONUCLEAR CELLS BY FLOW CYTOMETRY.

M. G. Wing, D. F. Lanham, E. L. Hunter and J. M. Bidgood. *Experimental Biology, Huntingdon Life Sciences, Huntingdon, United Kingdom.* Sponsor: C. Atterwill.

Flow cytometry has become a recognised method to measure mononuclear cell numbers on samples taken from toxicology studies. For reasons of epitope expression and biopharmaceutical immunogenicity, non-human primates are often the toxicological species of choice. In the absence of reagents specific for non-human primates, commercially available cross-reactive anti-human monoclonal antibodies were evaluated in cynomologus monkeys and marmosets, using multiple colour analysis to improve the accuracy of immunophenotyping and reduce blood requirements. EDTA-blood was stained with cocktails of monoclonal antibodies then the human and cynomolgus samples lysed with TQ Prep (Beckman Coulter) or Pharmlyse (BD Pharmingen) for the marmoset samples. The non-human primate samples were analysed for total T lymphocyte (CD3+) and B lymphocyte (CD20+) numbers by flow cytometry using cell scatter to identify the lymphocyte population. T lymphocyte subset analysis was performed using a CD3 T-gate. The principle difference noted was the higher number of CD4 and CD8+ T lymphocytes and B lymphocytes in the cynomologus monkey which were 2-3 times higher than that seen in the marmoset and human samples. The cynomolgus monkey also had a higher proportion of peripheral blood B lymphocytes (~25%) compared to the common marmoset and man (~15%). In conclusion, 3-colour immunophenoptyping panels have been successfully used to analyse marmoset and cynomolgus monkey peripheral blood. Good similarity was noted between the human and non-human primate parameters, and in particular between the common marmoset and man.

1167 HUMORAL IMMUNE RESPONSE TO KEYHOLE LIMPET HAEMOCYANIN (KLH) IN THE CYNOMOLGUS MONKEY.

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The safety assessment of immunomodulatory biopharmaceuticals require immunopharmacology/toxicity studies to be performed in a pharmacologically active species. Because species selection may have to take account of restricted epitope expression, ligand specificity and immunogenicity of human proteins, the non-human primate may be the most relevant species. Accordingly, the humoral immune response to the T-dependent antigen KLH was performed in the cynomolgus monkey to monitor specific antibody production. Groups of cynomolgus monkeys were immunised with KLH using 3 different protocols, then specific antibody to KLH was measured using a capture ELISA. In protocol 1, the animals received 2x100ug of KLH i.d. 43 days apart. Primary and secondary antibody responses were observed in all animals, however the variability in the response suggested that the dose of antigen or its formulation were not optimal. In protocol 2, the time between the two immunisations was reduced to 14 days to fit the procedure into a 4-week regulatory toxicology study. The animals received 2x100ug of KLH i.d. with the first dose formulated in Freund's incomplete adjuvant. This resulted in a robust and homogeneous antibody response, however the primary and secondary antibody responses were merged. In protocol 3 the animals received 2x1000ug KLH s.c. 14 days apart. Again the antibody response was homogeneous and robust, plus the primary response which typically plateaued by day 15, was distinguishable from the secondary response. In conclusion, protocol 3 offered the advantage of producing a homogeneous response without the need to use adjuvant and importantly allowed the primary and secondary responses to be monitored within a 4-week regulatory toxicology study design.

1168 ANALYSIS OF RAT IMMUNOTOXICOLOGY, HEAMATOLOGY AND CLINICAL CHEMISTRY PARAMETER VARIABILITY.

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Common methodologies are cited in regulatory guidelines for the immunotoxicity testing of pesticides, industrial chemicals, food additives and NCE's. These include immunophenotyping by flow cytometry, natural killer (NK) cell activity in a chromium release assay and specific antibody formation in a plaque forming cell (PFC) assay. Concerns have been raised over the variability seen in these assays and so immunotoxicology data were compared with clinical chemistry, haematology and organ weight data from a series of rat studies. Using data from control or unaf-

fected groups, the pooled within-group coefficient of variation (cv) was estimated using analysis of variance on logarithmically-transformed data. The cv's for the various endpoints in the two functional assays, PFC and NK, were quite high at 60-65%. For flow cytometry, however, the cv's for the various cell-types averaged around 40% for absolute counts and 20% for percentage counts. Whilst many chemistry parameters and the large organs have cv's below 20%, some enzymes, differential cell counts and small organs (endocrine and reproductive) have cv's between 30% and 70%.The higher cv seen with the PFC and NK assays *versus* flow cytometry is most likely due to the multiple sample processing steps and in the case of the PFC assay, immunisation of the animals. In conclusion, although the functional assay data was shown to have a high inter-study variability, this was not substantially different to some of the haematology and clinical chemistry parameters routinely measured on toxicology studies. The position for flow cytometry was even more favourable.

1169 IDENTIFICATION OF THE AGE AT WHICH IMMUNOTOXICOLOGICAL PARAMETERS CAN BE EVALUATED IN RATS.

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Development of the immune system begins early in gestation and continues to mature through puberty. Exposure to immunotoxic agents may occur at any point in development (i.e., prenatal, early post-natal, juvenile). Numerous immune function assays have been used to identify immunotoxicity in adult rats, however, these assays have not been adequately tested for application during early post-natal and/or juvenile immune system development. The objective of this study was to determine validity of utilizing adult immunotoxicological assays on juvenile Sprague-Dawley rats. Animals were tested for contact hypersensitivity (CHS) response to 2, 4-dinitro, 1-fluorobenzene (DNFB), delayed type hypersensitivity (DTH) responses to bovine serum albumin (BSA), and primary immune response, as demonstrated by increases in anti-sheep red blood cell (SRBC)-specific IgM. Sensitization or immunization was initiated at 3, 4.5 or 6 weeks of age. At 3 weeks of age both males and females demonstrated elevated CHS responses to DNFB, (i.e., 52.8% and 55.1% increase over naive controls, respectively). In contrast, DTH response was absent in males, and only minimally increased in females. Anti-SRBC IgM production, as determined by ELISA, averaged 4.3 and 7.9 µg/ml for males and females (adult concentrations average 30 µg/ml). At 4.5 weeks of age, the CHS response was 14% and 7% over control for females and males, respectively. IgM production averaged 15.3 µg/ml in males, and 10 µg/ml in females. DTH response increased 16% in males and 9% in females. At six weeks of age (i.e., the onset of puberty), IgM concentrations were 17.9 µg/ml in males, and 46.9 µg/ml in females. DTH response showed a 16% and 14% increase in males and females, respectively, and the CHS response in males and females increased 62% and 36%, respectively. These results indicate while the immune function responses employed produced measurable reactions in these three experimental procedures, the protocols must be refined for testing juvenile animals. (This abstract does not reflect EPA policy.)

1170 VALIDATION OF A METHOD TO DETERMINE RELATIVE PROPORTIONS OF ERYTHROID, MYELOID AND LYMPHOID PRECURSORS IN RAT BONE MARROW BY FLOW CYTOMETRY.

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To assess toxicity in the bone marrow, we have validated an assay to determine the proportions of myeloid, lymphoid and erythroid cells in rats using flow cytometry based on the method described by A Saad et al, "Differential Analysis of Rat Bone Marrow by Flow Cytometry" Comparative Haematology International (2000) 10:97-101. Bone marrow cells from rat femurs were stained with fluorochrome conjugated antibodies against CD71 and CD45. Cells were acquired using a flow cytometer and gated according to their expression of CD71 and CD45. Cells with a high level of CD71 expression were regarded as erythroid. A gate was placed around the remaining myeloid and lymphoid cells and these two populations were distinguished according to their side scatter properties. Cell proportions were calculated as a percentage of the total bone marrow population. Parameters examined in this validation were intra- and inter-assay precision, reference range and stability of fixed samples. Results for both the intra- and inter-assay precision produced CVs within the acceptance criteria of ¡Ü20%. Reference range results showed the proportion of erythroid cells ranged from 31.27 to 38.33% in males and 32.88 to 43.21% in females. Myeloid/lymphoid cell proportions varied between 32.83 and 42.30% for males and 27.67 to 41.31% for females. When a gate was set only on the myeloid/lymphoid population, the proportion of myeloid cells represented 45.36 to 53.88% in males and 44.11 to 52.25% in females. The proportion of lymphoid cells ranged from 40.42 to 50.35% for males and 44.21 and 50.21% in females. Results for stability were rejected as the isotype controls at 48 and 72 hours displayed a high level of fluorescence. Intra- and inter-assay precision met the acceptance criteria and the reference range for males and females was determined. Stability was not acceptable at 48 and 72 hours, therefore samples must be analyzed within 24 hours of staining.

1171 FURTHER INTERGRATION OF THE MOUSE LOCAL LYMPH NODE ASSAY.

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The LLNA has been extensively characterized and is now accepted as a "stand alone" to the traditional guinea pig models to assess the sensitization potential of chemicals. The goal of this investigation was to facilitate the continued integration of the LLNA. First, we have begun using an approach whereby a test material (TM) is evaluated for primary irritation in a pre-screen, range finding study. Briefly, at least six concentrations of TM are applied to the ears of mice for two days and ear swelling is determined 24 hrs later. Based upon measurable, detectable elevations in ear thickness, and reported increases in ear swelling for known irritants, we identify the concentration that produces 10% ear swelling as the minimal irritating concentration (MIC). The concentrations used in the subsequent LLNA are based on the MIC. The validity of this method is demonstrated with benzalkonium chloride (BC) and known irritants and sensitizers. Administration of the MIC for non-sensitizers produces a stimulation index (SI) near or less than 3.0, the standard threshold for a positive LLNA. Second, the standard LLNA protocol calls for a 5-hour pulse with ^{3}H-thymidine. This amount of time is not consistent with what is known about cellular proliferation or the uptake of radiolabel. A reduction in pulse time would reduce the time of the assay and introduce flexibility in the design. Initial studies with strong sensitizers (5% TMA and 1% DNCB) indicated no significant differences in absolute dpms after a 2-, 3- or 5-hour pulse. However, the absolute dpms in the vehicle groups were decreased at the earlier timepoints; this ultimately had an impact on SI values. This trend repeated when BC or the mild sensitizer, HCA, were evaluated and resulted in SI that were slightly greater at 2-hours than at 5-hours despite similar dpm values following TM exposure. These studies indicate that a shorter pulse is possible, thus providing flexibility in the conduct of the LLNA. These studies also highlight the importance of using both SI and absolute dpms for the interpretation of the LLNA.

1172 TRITIATED THYMIDINE INCORPORATION AS AN ENDPOINT IN THE POPLITEAL LYMPH NODE ASSAY.

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The popliteal lymph node (PLN) assay is an investigational tool to predict drugs and chemicals that are likely to induce systemic autoimmune and immunogenic reactions in man. Typically, the endpoints used are lymph node weight and cellularity, but sensitivity is not considered optimal. In the present study, incorporation of tritiated thymidine was used. Groups of 8 Balb/c mice received 50 µl of 10, 20 or 40 mg/ml of streptozotocin, diphenylhydantoin, sulfamethoxa-zole, or metformin into one hind footpad. Two additional groups received either 20 mg/ml of imipramine or 50% ethanol. The same volume of the vehicle was injected into the other hind footpad. Seven days later they were sacrificed five hours after an intravenous injection of 20 µCi of tritiated methyl-thymidine. PLNs were weighed and the incorpo-ration of tritiated thymidine measured by liquid scintillation counting. PLN weight and thymidine incorporation were increased to a similar degree relative to the control limb with streptozotocin, diphenylhydantoin, imipramine and ethanol. No change was seen in either endpoint with sulfamethozaxole and metformin. Based on these results, the measurement of thymidine incorporation does not seem to enhance the sensitivity of the assay. In addition, it failed to identify either imipramine or ethanol as inducing a non-specific inflammatory response. Finally, the negative results obtained with sulfamethoxazole indicates that this assay will not readily detect immunogenic compounds.

1173 FURTHER EXAMINATION OF AN ALTERNATIVE VEHICLE FOR WATER SOLUBLE MATERIALS IN THE LOCAL LYMPH NODE ASSAY.

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Vehicle choice for the murine local lymph node assay (LLNA) is important and has been shown to influence the sensitization potential of haptens. Suggested vehicles include organic solvents and organic-aqueous mixtures. However, due to its poor

wetting qualities and high surface tension, water is not recommended so testing water soluble materials can be problematic. We previously identified a water-based vehicle that provided better skin wetting properties than water alone and assessed its performance in the LLNA relative to other solvents using two water soluble haptens, dinitrobenzene sulfonic acid and formaldehyde. This wetting vehicle, 1% aqueous Pluronic® L92 (L92) has been further examined using the metal salts potassium dichromate and nickel sulfate and its performance compared to dimethylsulfoxide (DMSO) and dimethylformamide (DMF). Estimates of the relative sensitizing potency in each vehicle were determined by calculation of EC3 values (the estimated concentration required to induce a threshold positive response). Potassium dichromate was positive in DMF, DMSO and 1%L92. The potency ranking was DMF ≅ DMSO > 1% L92 (EC3 values were 0.03%, 0.05% and 0.17%, respectively). Potassium dichromate in water was negative. Nickel sulfate induced threshold positive responses when formulated in DMSO and 1% L92 (stimulation indices of 3.1 and 3.0, respectively), whereas the maximal stimulation index recorded following application in DMF was 2.2. Based on the results of this study, we recommend either DMF or DMSO as the preferred vehicle for water soluble materials in the LLNA. However, if the chemical is not soluble in DMSO or DMF, or, if higher concentrations can be achieved in an aqueous vehicle, then 1% L92 provides a better alternative to water alone in terms of improved assay performance for hazard identification.

1174 USE OF A B CELL MARKER (B220) TO DISCRIMINATE BETWEEN ALLERGENS AND IRRITANTS IN THE LOCAL LYMPH NODE ASSAY (LLNA).

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It has been shown that mice exposed to contact allergens show selective B cell activation in the draining lymph nodes (DLN) as seen by an increase in the % of B220$^+$ or IgG/IgM$^+$ cells. We have now examined whether measurement of % B220$^+$ cells could be used as an alternative endpoint for the LLNA to differentiate between allergenic responses and those few irritants which induce low level proliferation in the DLN. Mice were treated on the ears for 3 consecutive days with concentrations of allergens or irritants known to cause proliferation in DLN cells. The DLN were excised 72 h after the final treatment and the cells were prepared for B220 analysis by flow cytometry. The % B220$^+$ cells from test and vehicle treated mice were determined for 5 allergens and 4 irritants in multiple experiments (n=3 to 17). As expected, the % of B220$^+$ cells was increased with the allergens tested, whereas the % B220$^+$ cells was unaffected by irritant treatment. The % B220 values for each chemical treatment (41 allergen and 28 irritant observations) *versus* the % B220 value for the matched vehicle control were plotted and a classification tree model was developed to calculate a B220 test/vehicle ratio cutoff of 1.25 for discriminating between allergens and irritants. Using this ratio, 93% of the allergens and irritants were classified correctly. Moreover, the method was very reproducible, with the strong allergen 1-chloro-2, 4-dinitrobenzene classified correctly in 17 of 17 experiments and the weak allergen α-hexylcinnamicaldehyde classified correctly in 12 of 13 experiments. To evaluate the performance of this model ina second independent laboratory, 3 allergens and 2 irritants were tested. Each of the allergens and irritants were classified correctly using the B220 test/vehicle cutoff of 1.25. These data demonstrate that analysis of B220 expression on B cells may be useful in differentiating allergen and irritant responses in the DLN of chemically treated mice.

1175 PRE-VALIDATION STUDY FOR THE POPLITEAL LYMPH NODE ASSAY AS A TOOL TO PREDICT THE IMMUNOSENSITIZING CAPACITY OF (NON-) PHARMACEUTICALS.

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A large number of pharmaceutical drugs and non-pharmaceutical chemicals are known to cause systemic hypersensitivity and autoimmune-like clinical phenomena. The multifactorial pathogenesis of chemical-induced hypersensitivity is a major problem in developing models that allow recognition of the immunosensitizing potential of chemicals. To date no validated and generally applicable predictive immunotoxicological screening methods are included in traditional toxicity testing. The (reporter antigen-) popliteal lymph node assay (RA-PLNA) is regarded as a promising tool for this purpose as the RA-PLNA in particular is able to discriminate between compounds that act as adjuvant without stimulating specific T cell responses and chemicals that stimulate neo-antigen specific T cells. So far this assay has not been validated. We studied specific immune responses in the RA-PLNA and compared those with responses in the auricular lymph node (ALN) after s.c. injection of the drug together with the RA TNP-Ficoll into the ear (importantly s.c. injection between the ears did not elicit a TNP-specific response in the ALN). Responses showed similar proliferation of Th, Tc and B cells in the assays but the increase in dendritic cells was higher in the RA-PLNA. TNP-specific antibody responses were also more increased in the PLN than in the ALN. Clearly, results reveal that both assays, RA-PLNA and "RA-LLNA" are capable of identifying sensitizing chemicals (D-penicillamine, hydantoin, streptozotocin, ofloxacin), but that the RA-PLNA is more sensitive. Additionally, the narrow s.c. space in the ear requires injection of smaller volumes thus limiting the amount of chemical that can be injected. This also causes difficulties with injection of non- or poorly soluble chemicals and evidently affects reproducibility. Together, this study contributes to the validation of the RA-PLNA as a predictive test for immunosensitizing capacity of chemicals and indicates the advantage of footpad injection over ear injection.

1176 THE LOCAL LYMPH NODE ASSAY: CURRENT REGULATORY STATUS.

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The local lymph node assay (LLNA) is an alternative to guinea pig tests for identification of skin sensitising chemicals. In the standard LLNA protocol, CBA strain mice receive 3 consecutive daily topical applications of test substance (at 3 dose levels). Five days after the initiation of exposure, proliferative responses in lymph nodes draining the application site are measured by radiolabelled thymidine incorporation *in situ*. Chemicals which induce proliferative responses ^{3}3x those observed in concurrent vehicle treated control animals are considered to be skin sensitisers. The assay has been endorsed as a stand alone method for the determination of contact allergenic hazard by the FDA, the EPA and others following approval by the Interagency Co-ordinating Committee on the Validation of Alternative Methods (ICCVAM). The LLNA has also received formal approval from the European Centre for the Validation of Alternative Methods (ECVAM). Following these formal validations, the UK submitted the LLNA as an alternative method to the Organisation for Economic Co-operation and Development (OECD). The application has now been through redrafting and detailed discussion by the member states. It was approved by National Representatives in Paris on May 1st 2001 as OECD Guideline 429 and has been submitted to the Joint Meeting for final approval. Discussions are now underway concerning how to incorporate the LLNA into regulatory texts within the EU and within the Globally Harmonised System of toxicological testing.

1177 PREDICTION OF HUMAN SENSITISATION POTENCY USING LOCAL LYMPH NODE ASSAY EC3 VALUES.

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Effective evaluation of skin sensitisation demands that potential contact allergens are identified and the risk of sensitisation amongst exposed populations assessed. Sensitisation hazard is not an all or nothing phenomenon; Dose-Response relationships can be discerned and thresholds identified for induction and elicitation of contact allergy. These parameters, under the heading of potency, are vital for risk assessment. The murine local lymph node assay (LLNA) is an accepted method for the identification of sensitisation hazards (a sensitiser is a chemical that induces a stimulation index 3 or more times that of the concurrent vehicle control). The LLNA has also been used to determine the potency of sensitisers, by derivation of an EC3 value, the estimated concentration of chemical required to induce a stimulation index of 3. Here we have compared potency assessments derived from LLNA data for 40 chemicals which have been classified as strong, moderate, weak, extremely weak or non-sensitisers on the basis of clinical information. The potency of each chemical was also independently classified, based on its LLNA EC3 value. The two sets of potency rankings were then compared. The potency ranking of the allergens was very similar using EC3 values or human clinical experience, with 36/40 being correctly classified by the LLNA. A good correlation existed across all of the potency classifications. Importantly the 21 chemicals evaluated and classified as sensitisers for humans and which would merit regulatory classification were categorised identically by the LLNA. This investigation demonstrates that the LLNA can provide quantitative estimates of relative skin sensitising potency which correlate well with human potency. The data support use of EC3 values for estimating skin sensitisation potency and the use of that information in the risk assessment process.

1178 RESTRAINT STRESS MODULATION OF EAR SWELLING IN ACD IS INDEPENDENT OF SENSITIZING DOSE OF CHEMICAL.

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Stress plays an important role in numerous chronic health problems including modulation of immunologic diseases, however, the interaction between the stress response and the cutaneous immune response are incompletely understood. We have previously shown that acute stress suppresses the immune response in allergic contact dermatitis (ACD), and additional studies have shown a significant interaction between the concentration of chemical required for sensitization and for challenge in the development of ACD. We hypothesized that 2 hour restraint stress would change the sensitizing concentration of chemical required to elicit a full ear swelling response to chemical challenge. Male BALB/c mice were exposed on the back with increasing doses (0.05%-0.5%) of 2, 4 dinitrofluorobenzene (DNFB) on days 1 & 2 and challenged onto the ear with 0.1% DNFB on day 6. Mice were restrained for 2 hours prior to chemical application on day 1. We assessed ear swelling 24, 48 and 72 h post-challenge. Quantitative analysis of the data indicated that the ear swelling response to chemical challenge is dependent upon the sensitization concentration of DNFB and is dose-responsive. The curve shows a quadratic trend with 0.1% as a possible maximum dose for sensitization. Application of restraint stress did not alter the shape of this curve but significantly suppressed ear swelling at all concentrations of DNFB. These data suggest that restraint modulation of ACD is independent of the sensitizing dose of chemical.

1179 EXPRESSION OF TH2 CYTOKINES IS A GENERAL FEATURE OF MURINE CONTACT ALLERGY.

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The cytokine response pattern following sensitisation (induction) and elicitation (challenge) of contact allergy in BALB/c mice with different chemicals (dinitrochlorobenzene, dinitrofluorobenzene, oxazolone, glutaraldehyde, formaldehyde, trimellitic anhydride, croton oil) was investigated. The results of our investigations showed that contact allergens induced both T helper (Th) 1 cytokines interleukin (IL) 2, interferon gamma (IFNg) and Th2 cytokines (IL-4, IL-10) at different stages during murine contact allergy. We also confirmed our previous findings that IL-4 and IL-10 release were up-regulated during the challenge phase regardless the contact allergen used, whereas the release of IFNg did not show a clear preference for being up- or down-regulated. In our hands, the increased expression of Th2 cytokines after challenge exposure to contact allergens appeared as a stable marker of secondary contact allergenic responses. Quantitative differences in the expression of IL-4 were observed between different contact allergens. The present results clearly indicate that skin sensitisers were able to elicit cytokine response patterns, which could not be related to a clear-cut Th1 or Th2 type of cytokine response. Furthermore, topical application of contact allergens produced different kinetics of cytokine secretion upon induction and challenge. In our hands, the co-expression of Th1 and Th2 type cytokines appeared as a universal consequence of dermal application of contact allergens to responsive mice. Our results also indicate that chemicals differ in their potency to induce the expression of these cytokines. Furthermore, the results do not support the view that different chemicals induce Th1 or Th2 cytokines in a mutually exclusive manner depending on their preference to induce either contact or respiratory allergy. The results are expected to renew the discussion about the usefulness of the Th1/Th2 paradigm in certain areas of immunotoxicology.

1180 THE USE OF CD54 AND CD86 EXPRESSION ON THE HUMAN THP-1 AND KG-1 CELL LINES TO PREDICT CONTACT SENSITIZERS.

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In regard to *in vitro* skin sensitization tests, dendritic cells (DCs) derived from human peripheral blood have been considered in the development of new tests. However, there are some problems with the use of human DCs in skin sensitization tests. These include availability of human blood, donor to donor variability and other problems. In the present study, we evaluated the use of two human cell lines, THP-1 (monocytic leukemia cell line) and KG-1 (acute myelogenous leukemia cell line) in developing a method to identify contact sensitizers. Naive cells were used in our studies after comparing the usefulness of cytokine-derived cells and naive cells. The expression of CD54 and CD86 on THP-1 and KG-1 cells was measured using flow cytometry after a 24-hour exposure to known allergens (e.g., DNCB, 2-MBT, $NiSO_4$) and non-allergens (e.g., SLS, methyl salicylate, DMSO). We found that only the allergens enhanced the expression of CD54 and/or CD86 significantly in a dose dependent manner. In particular, metal salts, such as $NiSO_4$ and $CoSO_4$, increased only the CD54 expression. Additionally, we examined the signaling pathways of DNCB in order to study the intracellular mechanisms involved in the activation of THP-1 by DNCB. Results obtained using Western blotting showed that the phosphorylation of p38 MAPK was increased after 30-minute exposure to DNCB. Furthermore, SB203580, a specific inhibitor of the p38 MAPK, inhibited the up-regulation of CD54 and CD86 upon stimulation with DNCB. These results suggested that the up-regulation of CD54 and CD86 was coupled to the phosphorylation of p38 MAPK. This method was further evaluated using more than 20 of our materials. The expression of CD54 and CD86 on THP-1 and KG-1 cells used for predicting the sensitization potential of these materials was compared to guinea pig test results. A higher accuracy was observed with THP-1 (>70%) than with KG-1 cells. These results suggest that *in vitro* sensitization tests using these cell lines, especially THP-1 cells are useful to predict various contact sensitizers.

1181 INTEGRATION OF THE PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) ASSAY INTO STUDIES TO IDENTIFY POTENTIAL CHEMICAL ALLERGENS.

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Establishing a predictive test to identify chemicals that may induce respiratory sensitization is a pressing need in safety assessment. Concerns over the introduction of novel protein allergens has resurrected an interest in the PCA assay to measure antigen-specific IgE. This study compared the ability of the PCA assay to identify known protein (ovalbumin; OVA) and chemical (trimellitic anhydride; TMA) respiratory sensitizers. Balb/C mice were systemically sensitized to OVA *via* two i.p. injections seven days apart with 5% OVA in PBS (or with PBS alone; control). Test serum was collected 21 and 28 days following initial exposure. Serial dilutions of test sera were injected i.d. into the ears of Balb/c mice or the shaved backs of Sprague-Dawley (SD) rats. OVA and Evan's blue dye were injected i.v. 48 h later. The lowest dilution producing a positive reaction (≥ 2 x 2 mm spot) was recorded. Both heterologous and homologous PCA assays indicated that Day 28 OVA serum had the highest titer. For the chemical allergens, A/J mice were treated topically with 25% TMA, 1% dinitrochlorobenzene (DNCB; dermal sensitizer) or acetone:olive oil (AOO; vehicle) on their shaved backs. Seven days later, 12.5% TMA, 0.5% DNCB, or AOO was topically applied to the ears. Serial dilutions of test sera, collected 7 days later, were injected into the ears of A/J mice or the shaved backs of SD rats. Four hours later, 12.5% TMA or 0.5% DNCB was topically applied at the injection sites and 0.5% Evan's Blue dye was injected i.v. Only mice injected with TMA sera and challenged with TMA displayed a positive PCA reaction. ELISA confirmed the presence of a modest increase of total IgE in the DNCB serum, a marked increase of total IgE in TMA serum and no elevation of IgE in AOO serum. The timing of serum injection, test chemical application, and assay endpoint must be optimized. These data suggest that the PCA assay may be used to identify elevations in specific IgE induced by chemicals which may induce respiratory sensitization.

1182 CYTOKINE PROFILING FOR CHEMICAL SENSITIZERS: EFFECT OF DOSE.

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Chemicals encountered in both domestic and occupational settings may result in airway hypersensitivity (AHS). Because cytokine profiles generated during a T cell response are indicative of AHS (Th2) or contact hypersensitivity (CHS) (Th1), cytokine expression in draining lymph nodes (LN) has been used to identify chemicals with AHS potential. In this study, female BALB/c mice were sensitized twice on the flanks and challenged 3 times on the ears using the airway sensitizer trimellitic anhydride (TMA), or the contact sensitizer dinitrochlorobenzene (DNCB). Doses were varied during either: sensitization, challenge or both simultaneously. At various times following challenge, total mRNA was isolated from draining LN and analyzed by ribonuclease protection assay (RPA). When dose varied during sensitization only, TMA induced higher levels of the Th2 cytokines IL4, IL10 and IL13 than DNCB. However, no dose-dependent responses were observed with either chemical. DNCB did not induce Th1 cytokines at any dose tested. Variation of TMA dose during both sensitization and challenge also induced IL4, IL10 and IL13, and again showed no dose dependency. Dose only appeared to be a factor when TMA concentration was varied during challenge alone, suggesting the magnitude of the response depends upon the challenge phase. Thus, dose appears not to affect quantitative differences in Th2 responses between TMA and DNCB. These results suggest conventional cytokine profiling studies in which the same dose is

used for sensitization and challenge, allow the detection of differential cytokine responses regardless of chemical concentration. Lack of an IFN*gamma* response to DNCB may be due to the Th2-prone nature of the BALB/c mouse; therefore studies are currently underway to evaluate cytokine mRNA levels in Th1-prone C57BL/6 mice. (This abstract does not reflect EPA policy. This work was supported in part by The Dow Chemical Co. & DuPont Co.)

1183 CYTOKINE PROFILING FOR CHEMICAL SENSITIZERS USING THE RIBONUCLEASE PROTECTION ASSAY: DETERMINATION OF CYTOKINES GENERATED BY ISOCYANATES.

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Certain diisocyanates and acid anhydrides, which have been associated with occupational asthma, appear on the Hazardous Air Pollutant list (Clean Air Act 1990). Recent reports suggest that respiratory sensitizers may be identified based on their ability to induce a cytokine profile characteristic of a Th2 T cell response. Previous studies from this laboratory have shown that RNA message levels for the Th2 T cell cytokines IL4, IL10 and IL13 are elevated in response to the respiratory sensitizer trimellitic anhydride (TMA) and these increases may be detected by ribonuclease protection assay (RPA). In the current study, 4 additional chemicals (toluene diisocyanate (TDI), diphenylmethane-4, 4'-diisocyanate (MDI), dicyclohexylmethane-4, 4'-diisocyanate (HMDI) and isophorone diisocyanate (IPDI)) have been evaluated for their ability to induce Th2 cytokine mRNA expression. TDI and MDI are known respiratory sensitizers; however, there is conflicting data in the literature concerning the respiratory sensitizing potential of HMDI and IPDI. In the studies outlined here, female BALB/c mice were topically exposed to each of the isocyanates, and total mRNA was isolated from draining lymph nodes (LN) 14 days post challenge. RPA analysis showed that all 4 isocyanates tested induced cytokines characteristic of a Th2 T cell response (IL4, IL10 and IL13). These data support previous studies indicating that HMDI and IPDI are in fact respiratory sensitizers. (This abstract does not reflect EPA policy. This work was supported in part by The Dow Chemical Co. & DuPont Co.)

1184 AIRWAY RESPONSES AFTER SPECIFIC CHALLENGE OF RATS SENSITIZED *VIA* SKIN EXPOSURE TO TRIMELLITIC ANHYDRIDE (TMA).

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TMA is a low-molecular-weight chemical that can induce production of specific IgE and occupational asthma in sensitized individuals. The respiratory tract is considered to be a major exposure route leading to immunological and airway sensitization, but the relationship between dermal exposure and subsequent airway responses to TMA is not known. Our previous work, using Brown Norway (BN) rats, demonstrated that topical skin exposure to dry TMA powder induces dose-dependent production of specific IgE. The present study investigated the airway responses to inhaled TMA of BN rats that have been sensitized dermally with TMA. Twenty mg of dry TMA powder was applied to the rat's back (clipped with scissors) on days 0, 7, 14 and 21, and occluded overnight with surgical tape. Rats were challenged by a 10 min, 40 mg/m3 TMA aerosol inhalation on day 35. Enhanced pause (Penh), an index of airway narrowing, was recorded overnight in a whole body plethysmography system. Compared to non-sensitized rats, the sensitized BN rats displayed both distinct early (EAR) and late airway responses (LAR), as noted by an increase in Penh after TMA inhalation. The EAR occurred immediately following the challenge and lasted approximately 0.5 to 1 hour. This was followed by 2 hours of normal breathing. The LAR began from 3 to 4 hours after challenge and the increase in Penh lasted up to 19 hours following exposure. This work and our previous studies demonstrate that dermal exposure to TMA powder can lead to both immunological sensitization and obstructive airway responses upon aerosol challenge. This BN rat model, with both EAR and LAR, may be valuable for further study of pathophysiological mechanisms of organic acid anhydride-induced asthma.

1185 CHEMICAL AND VEHICLE RESPONSES TO TRIMELLITIC ANHYDRIDE (TMA) AFTER INTRATRACHEAL (IT) CHALLENGE OF SENSITIZED MICE.

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Exposure to some low molecular weight (LMWT)chemicals has been known to produce respiratory hypersensitivity (RH), especially in occupational settings. Methods to predict the RH potential of new chemicals are needed by the regulatory community. While mouse models for RH to proteins are available, mouse models for LMWT chemicals have not been developed. Our goal was to assess the effect of IT-exposure of TMA (free or conjugated) in selected vehicles. We evaluated lung responses to the known respiratory sensitizer TMA by dermally sensitizing and IT challenging mice. The contact sensitizer, 2, 4-dinitrofluorobenzene (DNFB) was the negative control. Abdomens were exposed to 25% TMA for 2 consecutive days for 2 weeks. In week 3, TMA was applied to the ears. Two weeks later mice were IT-exposed to: TMA in Hanks' Balanced Salt Solution (TMA-HBSS), TMA in olive oil (TMA-O), mouse serum albumin-TMA conjugate in HBSS (MSA-TMA). Serum and bronchoalveolar lavage fluid (BALF) were collected before (D 0) and 1, 2, 3, and 7 D post IT (PIT). TMA-sensitized mice challenged with TMA-HBSS had an increase in BALF total IgE at D1and D2. Serum total IgE increased in TMA-HBSS and MSA-TMA IT groups as compared to controls. While DNFB-HBSS challenge did not increase in serum total IgE, an increase in the MSA-DNFB challenged group suggests that once conjugated to a protein, even "non-respiratory" sensitizers may stimulate IgE. Serum IgE increased in agent-oil and oil alone at D 7 PIT in non-sensitized groups only, indicating that oil IT alone can evoke an independent serum total IgE increase. Our results suggest that none of these methods for delivering TMA to the lung were entirely satisfactory. It is clear that attention is needed in the area of chemical structure, stability, solubility, chemical- vehicle compatibility and compatibility with the biological system in order to develop appropriate models for the evaluation of LMWT chemicals for RH potential. (This abstract does not reflect EPA policy.)

1186 CD11C/CD1A POSITIVE DENDRITIC CELLS FOR THE IDENTIFICATION OF CONTACT SENSITISERS.

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The use of human blood derived dendritic cell (DC) cultures has been examined as an *in vitro* alternative for the identification of contact sensitisers, although often with variable outcome and not always with evidence of specificity. Thus we aimed to derive a purified 'Langerhans cell (LC) like' DC population, a cell type more relevant for the identification of skin allergens. Pure populations of DC were isolated from human peripheral blood using BCDA-1 magnetic bead separation. The cells were cultured for 5 days in a combination of granulocyte-macrophage colony stimulating factor, interleukin-4 and transforming growth factor-b to generate LC-like cells. The cells generated were approx. 95% CD11c/CD1a positive, expressed HLA-DR but not CD86 or CD83 as determined by flow cytometry, demonstrated endocytic ability (FITC-dextran uptake), were weak stimulators of the mixed lymphocyte reaction and thus could be regarded to possess an 'immature' phenotype. Cells obtained by this method (from either human peripheral blood or leucopacs) were exposed to subtoxic doses of the potent contact sensitiser DNCB (2, 4-dinitrochlorobenzene). This resulted in elevated expression of HLA-DR (2-7 fold increase in mean fluorescence intensity [MFI]) and CD86 (15-20 fold increase in MFI) compared with control cells or those treated with subtoxic doses of the irritant sodium lauryl sulphate. Culture of blood derived CD11c+ dendritic cells thus may provide a population of Langerhans like cells for potential future *in vitro* approaches to the identification of skin sensitisers. Further investigation will determine whether this approach will provide a robust predictive model that stands up to future challenges of specificity and sensitivity.

1187 EVALUATION OF ANTIGEN SPECIFIC IgE RESPONSES IN C3H/HeJ MICE EXPOSED WEEKLY BY ORAL GAVAGE FOR SIX WEEKS WITH COW'S MILK PROTEIN AND CHOLERA TOXIN.

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The objective of this study was to evaluate the antigen specific IgE Dose-Response in mice intragastrically exposed to cow's milk protein (CMP) and cholera toxin (CT) over a six-week period and to compare the responses to a cow's milk hypersensitivity model published by Li et al. (J Allergy Clin Immunol 103:206-214, 1999). Three-week-old female C3H/HeJ mice were sensitized by oral gavage to CMP plus CT and boosted 5 additional times at weekly intervals. Sera were collected at weekly intervals and the level of casein specific IgE measured by ELISA. Test groups (n=7 to 8) included naïve controls, 0.1 mg/g CMP + CT, 1 mg/g CMP + CT, 2 mg/g CMP + CT, 1 mg/g CMP without CT, and a CT only control. CT was administered at 0.3 µg/g. Casein specific IgE concentrations were determined by comparing individual or pooled serum samples to a mouse IgE anti-DNP standard curve. All analyses were performed in duplicate. As reported by Li et al., the maximal response occurred in the 1.0 mg/g CMP + CT group. However, differences in several measurements also occurred. We observed statistically significant differences in casein specific IgE levels between the 1.0 mg/g CMP + CT group and

the control groups by week 3 (Li et al. reported significant responses by week 6). Additionally, by weeks 4 through 6 casein specific responders were measured in all groups, including controls. The 1.0 mg/g CMP + CT group had the highest fraction of responders and the highest pooled casein specific IgE concentration. The number of responders and strength of response observed in the control groups illustrated that some animals within each group were generating significant IgE titers specific for casein. Following these unexpected results, we learned that the standard animal feed used in our facility contained a proprietary amount of casein as a dietary supplement. Our results generally support those reported by Li et al. and illustrate the need to strictly control the possible sources of protein exposure when conducting animal hypersensitivity models.

1188 NEGATIVE CONTROLS FOR PROTEIN ALLERGENICITY STUDIES.

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We have previously demonstrated immune and pulmonary responses typical of allergic responses in BALB/C mice exposed to fungal extracts of *Metarhizium anisopliae* crude antigen (MACA). MACA is composed of equal protein concentrations of the three component extracts from mycelium and conidia as well as inducible enzymes. Initial identification of the IgE inducing proteins by SDS-PAGE analysis suggested that the conidial extract does not contain allergenic proteins. In order to further explore the possibility of a non-allergenic extract we exposed BALB/C mice to 4 aspirations (ASP) of 10 µg of MACA, Conidia extract (Conidia), bovine serum albumin (BSA) (a weak or non-allergenic control) in 50 µl Hank's Balance Salt Solution (HBSS), or HBSS alone over a 4-week period. Serum and bronchoalveolar lavage fluid (BALF) were harvested before (D0), and at 1 (D1) and 3 (D3) days following the final ASP exposure. MACA exposed mice had elevated levels of both BALF total protein (D1 and D3) and lactate dehydrogenase (LDH) (D1). However, neither Conidia nor BSA exposed mice had increased levels of these non-specific parameters. BALF differential cells counts demonstrated that Conidia exposed mice had substantial increases in neutrophils, lymphocytes, and eosinophils compared to HBSS and BSA exposed animals. However, these increases represent low cell counts when compared to MACA exposed mice. Additionally, there was little or no increase in the levels of serum total IgE for either the Conidia or BSA exposed mice. These data suggest that the *M. anisopliae* conidia extract contains one or more proteins that cause mild inflammatory responses but would be classified as a weak or non-allergen. It appears that the conidia extract might be a useful negative control when assessing fungal extracts of unknown allergenicity in the mouse model. (Supported by NCSU/EPA Cooperative Training Agreement CT826512010.)(This abstract does not reflect EPA policy.)

1189 ANALYSIS OF PERIPHERAL BLOOD LYMPHOCYTE SUBSETS FROM COMMON MARMOSETS (*CALLITHRIX JACCHUS JACCHUS*) OF UK OR SWITZERLAND/JAPAN ORIGIN.

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The common marmoset (*Callithrix jacchus jacchus*) represents a recent addition to the primate species employed in preclinical safety studies in North America. The variability of lymphocyte subsets between marmosets with different geographical origins is unknown. Source-related differences in lymphocyte subsets were investigated using immunophenotypic analysis of peripheral blood by flow cytometry to quantify differences between common marmosets of UK or Switzerland/Japan origin. Peripheral blood samples were collected from the conscious restrained marmoset *via* the femoral artery or vein into trisodium citrate from 8 and 10 native UK and Swiss/Japanese marmosets, respectively. Two-color assays were utilized for lymphocyte subset analysis to measure total T-cells, T-cytotoxic/suppressor cells, T-helper cells, NK cells and B-cells. The procedure involved staining whole blood with monoclonal antibodies conjugated to two fluorochromes: CD20-fluorecein isothiocyanate (FITC), CD-3-FITC, CD-8-phycoerythrin (PE), CD-2-PE, CD-4-PE and CD16-PE. The samples were analyzed on a Becton Dickinson FACSCalibur®, a flow cytometer equipped with a 480 nm argon laser. Preliminary results suggest there is no statistically significant difference in the percent of total T-cells, T-cytotoxic/suppressor cells, T-helper cells or B-cells between the UK and the Swiss/Japanese colonies. However, significant differences were detected between the NK cells(CD16+/CD3-) from the two colonies. Since immune modulators may potentiate the NK lymphocyte subset differences in the UK and Swiss/Japanese marmosets, consideration of animal source-related differences in biophysiologic measurements is critical to study design.

1190 DEVELOPMENT OF AN ALTERNATIVE TEST FOR PHOTOIRRITANTS: THE *IN OVO* PHOTOTOXICITY ASSAY (IOPA).

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The potential phototoxicity of pharmaceuticals, consumer products and cosmetics are of increasing concern to regulatory agencies and the public. Preclinical phototoxicity studies are typically performed using large numbers of experimental animals. The 3T3 fibroblast phototoxicity assay has been validated by the EU, but this and other *in vitro* tests lack the complexity, metabolic capability and predictivity of animal models. We have devised an *In Ovo* Phototoxicity Assay based on the well-characterized chorioallantoic membrane(CAM) vascular assay, which uses the fertilized chicken egg as a test system. Groups of eggs were administered increasing concentrations of known irritants, non-irritants, and phototoxins, +/-5 J/cm^2 UVA irradiation. Eggs displaying vascular hemorrhage of the CAM, altered cell proliferation, or death of the embryo were scored as positive responses, and the percent positive eggs was plotted to determine the $EC_{50\text{-}UV}$ and EC_{50+UV} from the respective log-Dose-Response curves. The Photo-Irritancy Factor, (PIF = $EC_{50\text{-}UV}$ / EC_{50+UV}) was calculated and a PIF > 5 indicated a positive phototoxic response. Using this assay, the prototypical phototoxins 8-methoxypsoralen, promethazine, chlorpromazine, and 6-methylcoumarin were identified as phototoxic (8MOP; EC_{50+UV} = 0.0003 mg/ml > PMZ; 0.0017 mg/ml > CPZ; 0.082 mg/ml > 6-MC 0.44 mg/ml), whereas SLS, dinitrochlorobenzene, benzalkonium chloride, ethanol and mineral oil were negative. In addition, the IOPA correctly identified the metabolically-activated phototoxins nabumetone and 5-aminolevulinic acid, which are phototoxic *in vivo* (due to hepatic metabolism) but not when assessed *in vitro*. In these cases, up to an 18 hour pre-treatment time prior to UVA irradiation was necessary to allow for distribution and conversion to the phototoxic metabolite. In conclusion, we have developed an alternative phototoxicity assay that allows rapid, inexpensive and sensitive screening of chemicals, while retaining many advantages of animal models.

1191 ASSESSMENT OF OCULAR IRRITATION RANGES OF MARKET-LEADING COSMETIC AND PERSONAL-CARE PRODUCTS USING AN *IN VITRO* TISSUE EQUIVALENT.

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The cosmetics and personal-care industry has focused considerable effort on the search for replacements for traditional animal-based safety tests. *in vitro* models have been found to be particularly useful for the assessment of eye irritation potential. We have used the MatTek EpiOcular® OCL-200 tissue model (composed of human epidermal keratinocytes that differentiate and form a stratified squamous epithelium similar to corneal tissue) to establish ranges of *in vitro* ocular irritation scores for several categories of cosmetic and personal-care products. A significant advantage of the EpiOcular model is that it can be used to discriminate between the irritation potential of extremely mild products (most cosmetic and personal care products are formulated for inherent mildness). Materials were topically applied to the EpiOcular tissue equivalents (167µL/cm^2). Surfactant-based products were tested at a concentration of 10% to simulate "rinse-off" exposures; all other products were tested without dilution. Materials were tested using either the standard (4 hours) or extended (≅20 hours) exposure protocol based on their expected irritation potential (ie consideration of formula composition, product type, etc.). Cellular viability was used as a marker for irritation potential and measured at various time points by a MTT metabolism colorimetric assay. MTT was quantitated spectrophotometrically at 570nm and an ET_{50} (time to 50% loss of viability) calculated for each product. A range of *in vitro* ocular ET_{50} scores was then determined for each product category. The results obtained from this study provide a reference database of *in vitro* ocular irritation scores for a cross-section of currently marketed cosmetic and personal-care products. Comparison of the ET_{50} for new formulations with the range of ET_{50} scores established for currently marketed products of similar type is a useful benchmark of anticipated consumer acceptability under conditions of actual use.

1192 THE APPLICATION OF BENCHMARKS FOR THE EVALUATION OF THE POTENTIAL OCULAR IRRITANCY OF AEROSOL FRAGRANCES.

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Fragrances are complex mixtures used in many consumer products. In aerosol air care formulations, organic solvents, such as ethanol, are added to the final formulation as solubilizers, stabilizers, fillers, and fragrance delivery mechanisms. The current product development/safety program was established to preclude routine *in*

vivo ocular irritation testing and uses the Bovine Corneal Opacity and Permeability (BCOP) assay and reference formulations as "benchmarks" to calibrate the system and to establish acceptable ranges of irritancy potential. Abattoir-derived corneas were exposed to test materials for 3 or 10 minutes and opacity and permeability end points measured to calculate an *in vitro* score. These exposure times exceeded expected exposure times *in vivo* but were intended to increase separation among formulations. Negative (medium) and positive (100% ethanol) controls were tested concurrently with over 40 formulations (6 to 27% fragrance). The upper end benchmark induced an acceptable ocular response to a spray exposure *in vivo*. Ethanol alone produced an *in vitro* score of 52.4 ± 6.6 (10 minute exposure). The corneas showed surface coagulation of the squamous epithelium and marked nuclear swelling in the deeper epithelium. Changes in the upper stroma included moderate collagen matrix vacuolization (swelling) and keratocyte cytoplasmic eosinophilia. The addition of the fragrance component increased both parts of the *in vitro* score. The increase depended on the amount of fragrance (6 vs 27%) and the fragrance formulation itself. The upper end benchmark produced over double the *in vitro* score of ethanol alone. Histological changes were qualitatively similar but more pronounced. Acceptable new formulations would be expected to fall below the upper benchmark. These data show the need of using a formulation-specific benchmark to accurately assess ocular irritation in the *in vitro* BCOP assay, thus eliminating the need for *in vivo* testing for product evaluations.

1193 EXPLORATORY *IN VITRO* EYE IRRITATION STUDY OF MARKETED ALKALINE LIQUID LAUNDRY DETERGENTS BY BCOP ASSAY AND PH/RESERVE ALKALINITY (RA) PARAMETERS.

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An exploratory *in vitro* eye irritation study of 17 currently marketed alkaline liquid laundry (ALL) detergents was conducted to investigate the correlation between an *in vitro* biological endpoint and pH/RA ranges of ALL detergents. Currently marketed products can be considered "safe benchmarks", since they are produced by industry leaders and are assumed to have acceptable pH/RA characteristics. Based on performance in previous eye irritation studies with surfactants and the potential to measure depth of injury, the bovine corneal opacity and permeability (BCOP) assay was selected as an *in vitro* endpoint to evaluate biological effects. Based on preliminary studies, a 25% (w/v) aqueous dilution of each detergent was applied to the corneas for a 20-minute exposure period. The degree of epithelial damage is reflected in the increase in fluorescein permeability value. Permeability values (OD_{490}) for the 17 ALL detergents ranged from 0.278 to 2.193 reflecting a wide range in epithelial damage. The range of opacity scores was much lower (0.6 - 14.3) and showed little consistent change with the permeability values. Anionic/nonionic surfactant formulations often produce little *in vitro* opacity. The pH of each detergent dosing solution (25% w/v) was measured and ranged from 7.5 to 11.0. The RA (buffering capacity) was determined for a 1% (w/v) aqueous solution of each detergent titrated to a target pH of 9.5. Titration values ranged from 27.5 to 200 µmoles of NaOH or 10 to 120 µmoles of HCl. Neither pH nor RA values correlated with the epithelial damage as measured by permeability changes. These data suggest that characteristics of the formulation other than pH or RA are responsible for the epithelial damage produced in the BCOP. This BCOP protocol, using a 25% (w/v) aqueous dilution with a 20-minute exposure, the permeability endpoint (with histological confirmation) and benchmark formulations, shows promise for evaluating ALL detergents.

1194 USE OF HISTOLOGICAL EXAMINATION IN BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) ASSAY FOR ASSESSING THE OCULAR IRRITATION POTENTIAL OF FRAGRANCED FORMULATIONS.

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Fragrances are complex mixtures containing numerous chemical compounds and are referred to generically in the context of consumer product formulations. Aside from the inherently complex contribution of fragrance to a formulation's ocular irritation potential, it is also difficult to elucidate the role of other components in the mixture. This study was undertaken to evaluate the use of histological analysis to identify ocular damage not detectable by BCOP alone. Formulations were grouped according to base and fragrance type/concentration and comparisons made between base-only and a series of fragranced formulations using the same base. Ocular irritation was measured using a standard BCOP assay with isolated corneas exposed to test material for 10 minutes, followed by opacity and permeability determination and histological analysis. Ethanol was used as a concurrent positive control. Within a series, BCOP scores varied significantly across fragrance type, ranging from 0.1 to 44.8, due mainly to changes in permeability. Histological analysis confirmed the relative degree of damage. For a given base, predicted irritation potential was dependent on the particular fragrance mixture. This study reinforces the need to determine the ocular irritation potential of formulations and not rely solely on component information. Understanding the nature and degree of ocular irritation potential to complex mixtures may help to improve the selection process for base and fragrance components of consumer products.

1195 THE RABBIT ENUCLEATED EYE TEST (REET) AS A SCREEN TO REDUCE THE USE OF ANIMALS IN WORKER SAFETY STUDIES.

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Identification of chemicals that cause eye irritation in the work place has traditionally used the Draize eye protocol. The REET, an *ex vivo* alternative, shows promise for predicting severe eye effects. Test material is placed onto enucleated eyes (animals euthanized for other purposes) in chambers perfused with warm saline and washed off after 10 seconds. Corneal opacity, thickness and fluorescein dye uptake are measured and corneal surface examined. Thirty materials (pharmaceuticals, intermediates, raw materials) were tested in both the Draize and REET. The Draize test identified 8/30 materials as severe with an additional 5 identified based on pH/dermal effects. Overall, 13/30 chemicals were classified severe and labeled with EU phrase R41 - Risk of Serious Damage to Eyes. REET evaluation suggested that a cutoff score for any one of these parameters appeared predictive of the Draize response: corneal opacity/area >/= 3; corneal swelling >/= 25; fluorescein dye uptake/area >/= 4 or observations of pitting, mottling or sloughing. The REET-positive criteria were met by 17/30 materials and included 13 labeled R41 (100%) with 4 false positives (1 moderate, 3 mild). Thirteen chemicals did not meet the REET criteria: 9 were minimal/mild eye irritants and 4 were moderates. In conclusion, using the REET assay with pre-assigned cutoffs appeared to identify severe eye irritants and provided data for hazard labeling. When used as a pre-screen for the Draize assay, the REET contributes to reducing the number of animals used to test for eye irritants.

1196 DEVELOPMENT OF AN IMMORTALIZED Z310 CHOROID PLEXUS CELL LINE FOR DRUG TRANSPORT AND TOXICOLOGICAL STUDY OF BLOOD-CSF BARRIER.

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The blood-CSF barrier (BCB), located mainly in the epithelium of the choroid plexus, plays a wide range of roles in brain development, maturation, aging process, endocrine regulation, and pathogenesis of certain neurodegenerative diseases. To facilitate *in vitro* study of the BCB, we have used a gene transfection technique to immortalize murine choroidal epithelial cells. A viral plasmid (pSV3neo) was inserted into the host genome of primary choroidal epithelia by calcium phosphate precipitation. The transfected epithelial cells, i.e., Z310 cells, that survived from cytotoxic selection expressed SV40 large-T antigen throughout the life span, suggesting a successful gene transfection. The cells displayed typical polygonal epithelial morphology by light microscopy. Immunocytochemical studies of transfected cells demonstrate the presence of transthyretin (TTR), a thyroxine transport protein known to be exclusively produced by the choroidal epithelia. Western blot analyses further confirm the production and secretion of TTR by these cells. The mRNAs encoding transferrin receptor (TfR) were also identified by Northern blot analyses. The cells grow at a steady rate, currently in the 110th generation with a population doubling time of 20-22 h in the established culture. When Z310 cells were cultured onto a Trans-well apparatus, the cells formed an epithelial monolayer similar to primary choroidal cells, possessing distinctive features such as an uneven fluid level between inner and outer chambers and an electrical resistance approximately 150-200 ohm-cm^2. Moreover, the cells actively transported $[^{125}I]T_4$ across this monolayer. These results indicate that immortalized Z310 cells possess the characteristics of choroidal epithelia and may have the potential for toxicological research. (Supported by NIH/NIEHS RO1 ES08146)

1197 EVALUATION OF FETAL-RAT DORSAL ROOT GANGLIA (DRG) CULTURE SYSTEMS FOR ASSESSING CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHIES.

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A proposed mechanism for the induction of peripheral neuropathies involves effects on microtubule-dynamics, a process integral to neurite outgrowth (NG) and to the formation and maintenance of axon and dendrite terminals. For modeling nerve growth and arbor morphology, the sympathetic DRG isolated from fetal rodents can be cultured to promote NG. In this investigation, we evaluated fetal-rat, dissociated and whole-explant DRG culture systems using several oncolytics with distinct mechanisms of action including tubulin polymerization/depolymerization, DNA alkylation and topoisomerase inhibition. In the dissociated DRG model, cultures were prepared in 96-well plates and evaluated *via* fluorescence-labeling and automated image analysis (ArrayScan®). Whole-explants were prepared in multi-chambered slides and evaluated by light-microscopy and analyzed *via* Quantimet® image analysis. A viability assay was performed in each culture system to assess cytotoxicity. Both DRG models were employed to demonstrate concentration-dependent inhibition of NG in evaluation of: 1) compounds known to cause peripheral neuropathies in humans and 2) compounds not associated with neuropathic effects. In doing so, we showed that both test systems could be used to distinguish between specific and non-specific neuropathic agents by combining a viability assay with image analysis-techniques. The effects of various concentrations of nerve growth factor (NGF) were evaluated in both culture systems to demonstrate potential neuroprotection. In the whole-DRG model, we showed that elevated concentrations of NGF(>20 ng/mL) inhibited NG; as has been described previously. Interestingly, the dissociated-DRG culture system was relatively non-responsive to NGF concentrations between 2.9 and 900 ng/mL. Finally, we compared the relative merits of each culture system and the image analysis techniques carried out for both, and conclude that either system can be useful for evaluating compounds likely associated with peripheral neuropathy.

1198 EARLY ENDPOINTS FOR EVALUATING CADMIUM-INDUCED *IN VITRO* NEPHROTOXICITY.

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Cadmium chloride ($CdCl_2$) is a potent nephrotoxin that causes damage to the proximal tubular epithelium. *in vivo*, it increases the permeability of epithelial surfaces, while *in vitro*, it acts on active trans-epithelial ion transport. The purpose of our research was to investigate the effects of $CdCl_2$ on a porcine renal proximal tubular epithelial cell line (LLC-PK1). In particular, we aimed to study damage to the epithelial barrier (by measuring trans-epithelial resistance [TER] and paracellular permeability [PCP]), and the molecular mechanisms underlying $CdCl_2$ toxicity (by measuring metallothioneins [Mts], heat shock proteins [HSPs], reactive oxygen species [ROS], and the induction of apoptosis). After exposure of the cells to different concentrations of $CdCl_2$, TER decreased, while PCP increased in a concentration-dependent and time-dependent manner, indicating a structural alteration of the junctional complex. At the molecular level, we observed an increase in protective proteins, such as Mts and HSP70, starting from exposure to 10 µM and 25 µM $CdCl_2$, respectively. Production of ROS at the mitochondrial level was also evident, indicating cellular oxidative stress. Treatment of the cells with higher concentrations of $CdCl_2$ (100 µM) revealed a clear induction of apoptotic cell death. Our data indicate that $CdCl_2$ nephrotoxicity can already be detected at rather low concentrations by measuring TER (damage at the barrier level) or Mts, HSPs, ROS production (damage at the cellular level), when cytotoxicity assays are unable to show any real damage. Therefore, these endpoints should prove to be very useful in studying xenobiotic- induced acute toxicity.

1199 CULTURES OF EXFOLIATED HUMAN UROTHELIAL AND RENAL TUBULAR CELLS.

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Since tissues of the urinary tract or the renal tubule from surgery or transplant donors have very limited availability, a promising source for these cells is the urinary sediment. Midstream urine samples from 24 adults were concentrated to sediments by repeated centrifugation (100 x g, 5 min). To reduce urine concentration, sediments were washed twice with Ham's F12 culture medium. After resuspension in 0.5 ml culture medium, aliquots of 50-250 µl cell suspension were seeded onto collagen-coated 24-well plates. Cell culture was performed in a humidified incubator according to standard conditions. Vital cells started proliferation within 1 week after seeding and formed colonies of different morphology designated as type-1 or type-2 cell colonies. Most frequently, type-1 cell colonies occured which showed irregular colony contours and randomly arranged spindle-like cells. Subcultivation was possible up to 6 passages. Immunohistochemical staining for the epithelial marker cytokeratin-7 was positive. Staining for carbonic anhydrase was negative. Type-2 cell colonies which could be subdivided into two subgroups by morphological characteristics only occured occasionally. These colonies showed smooth-edged contours. A subcultivation was not possible. Cobblestone-like type-2 cells were able to form domes. These cells were positively stained for carbonic anhydrase, indicating a tubular origin. Primary cell cultures of exfoliated cells from urinary sediments can be used for *in vitro* studies in toxicological and clinical research.

1200 SUBACUTE CYTOTOXICITY TESTING WITH CULTURED HUMAN LUNG CELLS.

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This study was designed to evaluate the potential of an *in vitro* cell culture method for its ability to determine subacute cytotoxicity and to compare the cytotoxic concentrations with rodent LD50s and clinical human toxicity data. Human fetal lung fibroblasts (HFL1) were incubated in the absence or presence of increasing concentrations of test chemicals for 72 hours, and cell proliferation was used as a marker for toxicity. Inhibitory concentrations were extrapolated from concentration-effect curves after linear regression analysis. Comparison of the cytotoxicity data from testing 50 chemicals, with available human lethal concentrations for the same chemicals, revealed that the 72-hour experimental IC50s are as accurate predictors of human toxicity as equivalent toxic blood concentrations derived from rodent LD50s. In addition, subacute 72-hour exposure of HFL1 cells more accurately predicts cytotoxicity than a 24-hour mitochondrial assay previously conducted in our Lab., although the experimental IC50 values were not statistically different in the two assays (table below of first 15 chemicals). It is anticipated that this procedure, together with a related battery of tests, may supplement or replace currently used animal protocols to screen chemicals for human risk assessment.

Number	Chemical Name	72-hour IC50s: Cell Proliferation Assay	24-hour IC50s: MTT Assay
1	paracetamol	4.25±.5	7.76±.84
2	acetylsalicylate	1.3±.1	3.02±.76
3	ferrous sulfate	0.6±.1	-
4	diazepam	.67±.12	.28±.05
5	amitriptyline	.012±.002	.028±.004
6	digoxin	3.53±.20	1.95±.51
7	ethylene glycol	53.3±8.3	63.1±7.4
8	methanol	4.3±.8	41.7±4.2
9	ethanol	6.2±.35	25.1±3.3
10	isopropanol	7.3±1.0	5.8±1.1
11	1,1,1-TCE	1.5±.1	4.9±1.0
12	phenol	0.3±.0	1.3±.2
13	sodium chloride	5.3±.6	7.7±.9
14	sodium fluoride	.04±.01	.21±.03
15	malathion	.29±.06	.62±.08

1201 INHIBITION OF FATTY ACID ETHYL ESTER SYNTHASE ACTIVITY BY TRI-O-TOLYLPHOSPHATE METABOLITES IN HEPG2 CELLS.

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Fatty acid ethyl ester synthase (FAEES) is a family of enzymes that catalyzes the conjugation of alcohols and amines with fatty acids. Resultant conjugates are shown to be highly lipophilic, which can accumulate in target organs and cause toxicity. Previous studies from this laboratory have shown that FAEES purified from rat liver is structurally and functionally different from that of pancreas, and only hepatic FAEES activity is inhibited by tri-o-tolylphosphate (TOTP, a serine esterase inhibitor). Metabolism of TOTP is prerequisite for such inhibition. Therefore, two known metabolites of TOTP, 2-o-(cresyl)-4H-1:3:2-benzodioxaphosphoran-2-one (CBDP) and hydroxylated TOTP [di-o-tolyl-o-(α-hydroxy) tolylphosphate] were synthesized and their inhibitory effect on FAEES activity was investigated in HepG2 cells (human hepatoma cell line). HepG2 cells in culture were incubated with an equimolar concentration of 2.5 µM TOTP, CBDP or hydroxylated-TOTP

in ethanol at 37°C (a concentration causing 85% inhibition by CBDP in preliminary experiments). Cells treated with equivalent amount of ethanol served as control. Cells were harvested after 1 hour exposure, washed, scraped and homogenized. FAEES and p-nitrophenylacetate (PNPA) hydrolyzing (esterase) activities were measured. Inhibition of FAEES as well as esterase activities was found to be greatest in the cells treated with CBDP and hydroxylated-TOTP (~ 85 and 59%, respectively), followed by TOTP. The time course study performed at 0.25, 0.5, 1, 3 and 6 hours, demonstrated that inhibition by CBDP occurs in 15 minutes (earliest time point investigated). We conclude that the active metabolites of TOTP (CBDP and hydroxylated-TOTP) cause the inhibition of FAEES and esterase activities in HepG2 cells.

1202 MECHANISMS OF CERAMIDE-INDUCED APOPTOSIS IN A RAT HEPATOMA (H4IIE) CELL LINE.

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Ceramides are a group of sphingolipids that act as second messengers in the control of many biochemical functions including apoptosis. Tumor necrosis factor alpha (TNF) can increase the hydrolysis of sphingomyelin to form ceramide. Both ceramide and TNF have been shown to cause apoptosis *via* activation of caspases. Ceramide has also been shown to induce apoptosis *via* pathways independent of caspase activation. Thus, the precise signalling pathways activated by ceramide depend on the cell type. If TNF-mediated apoptosis is dependent on the formation of ceramide, then ceramide and TNF should produce similar effects *in vitro*. In the present study the biochemical pathways involved with ceramide-induced apoptosis were compared to those of TNF in a rat hepatoma (H4IIE) cell line. Cells were seeded into 96-well plates (10, 000/200 µL), allowed to equilibrate for 48 hr, and then exposed to C2-Ceramide (0, 0.1, 1, 5, 10, 50, 100, and 300 µM) or to TNF (0, 0.05, 0.5, 2.5, 5, 25, 75, and 150 ng/mL; specific activity = 1.0×10^9 U/mg) for 24 hr. Following the exposure period, changes in cell health were determined by monitoring mitochondrial function, membrane leakage, and cell proliferation. Oxidative stress was assessed by measuring changes in reduced glutathione (GSH) and membrane lipid peroxidation. Apoptosis was evaluated by caspase (3 and 9) activity and DNA fragmentation. TNF was cytotoxic above 5ng/mL, however, there were no significant changes in total GSH prior to cell death or in membrane lipid peroxidation as determined by 8-isoprostane. Exposure to TNF caused a marked increase in caspase 3 and 9 activities as well as DNA fragmentation, but did not increase lipid peroxidation. In contrast, ceramide had no effect on caspases 3 or 9, but did increase lipid peroxidation (10-300 µM ceramide). C2-ceramide also increased DNA fragmentation at the highest exposure (300 µM) and reduced mitochondrial function. These data suggest that in H4IIE cells apoptosis induced by TNF may occur through signalling pathways that are different from those induced by C2-Ceramide.

1203 HALOGENATED HYDROCARBON TOXICITY IN RAT PRIMARY HEPATOCYTES: INDUCTION OF OXIDATIVE STRESS AND DEVELOPMENT OF A NOVEL TOXICITY RANKING METHOD.

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Volatile halogenated aliphatic compounds are among those chemicals that can cause oxidative stress *in vitro* and *in vivo*. Here, we investigated the effects of 20 brominated and/or chlorinated one- or two-carbon molecules, including carbon tetrachloride, trichloroethylene and 1, 1, 1-trichloroethane. Following the use of a unique *in vitro* methodology to expose primary rat hepatocyte cells to these volatile chemicals, biological responses were assessed from separate exposures of rat primary hepatocytes to these halogenated aliphatics. Indicators of lipid peroxidation, reactive oxygen species, catalase enzyme activity, cellular thiol status, and cytotoxicity were measured. For this large group of chemicals, classification by multiple endpoints for oxidative stress in primary rat hepatocytes has not been shown previously. The toxicity values for these chemicals, such as the EC50s, ranged from 0.10 to 127.00 mM. We derived a novel composite toxicity index for purposes of ranking the potential toxicities of the chemicals. This scoring index had a potential range from 6 to 120, for ranking purposes. In this study, the composite scores ranged from 11 to 115. We also derived QSAR models to describe the relationships for these chemicals between their physico-chemical properties and their potential to cause oxidative stress *in vitro*. Our results show the ability to rank a group of chemicals, based on their *in vitro* toxicity values for multiple endpoints and the effectiveness of QSAR models in predicting relative toxicity ranking of chemicals. The impact of this approach provides a means of discerning the relative toxic potencies among a group of chemicals, which could be used in materials or drug development or identification of relative risk.

1204 RANKING GLITAZONE COMPOUNDS FOR HEPATOTOXIC POTENTIAL BY MONITORING STRESS-SPECIFIC ALTERNATIVE mRNA ISOFORMS: EVIDENCE AGAINST A GLITAZONE CLASS TOXICITY?

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A low incidence of severe unanticipated hepatotoxicity resulted in troglitazone (TRO) withdrawal. The ability of gene expression profiling in the Safe-Hit DNA macro-array, representing a library of 682 stress-specific p53-induced mRNA isoforms, to correctly rank for toxic potential three commercially available glitazone compounds was evaluated. Primary cultures of human hepatocytes from eight donors and the hepatocarcinoma line HepG2 were incubated for 16 hr with TRO, pioglitazone (PIO) or rosiglitazone (ROS) at concentrations ranging up to approximately 20 % cytotoxicity (MTT). The mRNA was isolated and used to probe the Safe-Hit macro-array. Up- or down-regulation of stress-specific mRNA isoforms by 2.5-fold relative to vehicle control cells was considered to represent gene deregulation, and a toxicity index corresponding to the total number of deregulated genes for that particular condition was calculated. TRO had the highest toxicity index in all cases and the difference was greater in the primary human hepatocytes than in HepG2. Toxicity indexes at the highest concentrations for TRO, PIO and ROS were 45, 11 and 7, respectively, in primary cultures of human hepatocytes and 33, 28 and 28, respectively, in HepG2 cells. The high toxicity index induced by TRO and the lower ones induced by PIO and ROS in human hepatocytes correlate with what is known about the clinical safety of these drugs and indicate that hepatotoxicity might not be a simple glitazone class-specific event. That the repertoire of deregulated genes differed between TRO on one side and PIO or ROS on the other further supports this idea. These results suggest that the monitoring of appropriately selected alternative splicing variants, such as those in the Safe-Hit library, may be a method of choice for *in vitro* detection of compounds that will induce rare severe liver toxicity, a phenomenon that historically has been difficult to predict.

1205 ANTIDIABETIC DRUGS IN THE THIAZOLIDINEDIONE CLASS REDUCE GLUTATHIONE LEVELS AND INCREASE LIPID PEROXIDATION IN AN *IN VITRO* MODEL.

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Thiazolidinedione (TZD), troglitazone (TRO), pioglitazone (PIO), and rosiglitazone (ROS) are in the TZD class of oral hypoglycemic drugs used in the treatment of diabetes. TRO has been shown to produce idiosyncratic liver toxicity. The mechanisms underlying TRO hepatic toxicity remain unknown. Metabolism of TZD drugs involves ring opening and subsequent conjugation with GSH. TRO has been shown to reduce GSH and induce oxidative stress. Patients with type II diabetes have a significant reduction in tissue GSH. Moreover, the ability of a tissue to protect itself from oxidative stress is dependent on many factors including diet, age, and exposure to other medications. Thus, some diabetic individuals may be unable to adapt to compounds that produce oxidative stress by depleting GSH. The purpose of the present study was to evaluate the relative cytotoxic and oxidative stress potential of TZD, TRO, PIO, and ROS in an *in vitro* cell system. Rat hepatoma (H4IIE) cells were seeded into 96-well plates (10, 000/200 µL). Following a 48 hr equilibration period the cells were treated with compounds at concentrations of 0, 0.1, 1, 5, 10, 50, 100, and 300 µM for 24 hr. Cytotoxicity was evaluated by measuring membrane leakage, mitochondiral function, and cell number. Oxidative stress was assessed by measuring GSH and 8-isoprostane (8ISP). Markers of general toxicity were essentially unchanged at exposures below 100 µM. TZD and PIO reduced GSH and produced a concomitant increase in membrane lipid peroxidation. These changes were observed at low concentrations prior to changes in general cell health. TRO also reduced GSH levels, but only produced a modest increase in 8ISP at the highest exposure. ROS reduced GSH, but had no detectable effect on 8ISP. Thus, in terms of oxidative stress: TZD = PIO >TRO > ROS. These data suggest that the antioxidant status of individuals with diabetes may be a determining factor in how they respond to the TZD class of drugs.

1206 INCORPORATING LC/MS/MS IN DETERMINING CYTOCHROME P450 FORM-SPECIFIC ACTIVITY.

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The goal of drug discovery organizations is to increase the probability of compound success in clinical trials. Currently, the three main reasons for clinical failure are lack of efficacy, unexpected toxicity and poor drug metabolism/pharmacokinetics. New

methods and technologies are constantly being incorporated into drug discovery to screen new chemical entities (NCEs) with regards to these three areas, especially in the area of metabolism. Metabolism screening techniques rely upon various *in vitro* test systems, such as microsomes, S9, hepatocytes, etc. In order to use these systems, the native activity of the drug metabolizing enzymes, primarily the cytochrome P450s (CYP450), must be determined. CYP450 activity is also used to determine the inhibition potential of NCEs. Inhibition of the NCE metabolizing enzymes could result in elevated blood levels and greater likelihood of toxicity. In order to increase screening throughput, a faster method to evaluate CYP450 activity was developed. Previous methods required incubating a CYP450 marker substrate with the test system and determining metabolite production *via* HPLC/UV detection. A new analytical method was developed using LC/MS/MS to evaluate the production of metabolites for six CYP450 forms (1A1/2, 2C9, 2C19, 2D6, 3A4/5 and 4A11) following either multiple incubations or a single CYP450 "cocktail" incubation. The inhibition potential of SK-203580 and VX-745 (0 to 25 μM) was determined using individual CYP450-specific substrate incubations as well as "cocktail" incubations of CYP450 marker substrates in pooled human liver microsomes. Regardless of incubation method, SK-203580 inhibited the activity of CYPs 2C19 and 2D6, and VX-745 inhibited the activity of CYP2C19 but not CYP2D6. Individual incubations with either SK-203580 or VX-745 decreased CYP3A4/5 activity but "cocktail" incubations with either compound had no inhibition towards CYP3A4/5.

1207 A SENSITIVE METHOD TO DETECT METABOLIC TOXICITY IN A HOMOGENEOUS CELLULAR ASSAY.

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A major concern in the pharmaceutical industry is the potential for metabolic toxicity of developmental compounds. However, existing assays are extremely cumbersome and time-consuming. Here, we present a cell-based, high throughput-screening assay that detects and differentiates between direct and indirect acting toxins. A B-lymphoblastoid cell line, MCL-5, was created expressing 5 human P450's known to be important in metabolic toxicity, CYP1A1, 1A2, 3A4, 2A6 and 2E1 and epoxide hydrolase. Direct and indirect toxicity was measured with the MCL-5 cells in comparison to its control cell line cH2 cells, using the BD Oxygen Biosensor System (OBS) as the method of detecting cell viability. Experiments were annotated and IC50's determined using the Multi-well Plate Manager (MPM) –ADMET software program. Data and the associated experiments were archived in MPMs' internal database for easy retrieval. The MCL-5 cells were shown to be significantly more sensitive to compounds that required metabolic activation for toxicity such as NNK, aflatoxin and benzopyrene (Table). The detection of toxicity for compounds such as acetaminophen required the inhibition of glutathione by 50uM buthionine sulfoximine (starred compounds*). Moreover, the metabolic toxicity of cyclophosphamide could be specifically inhibited by competitive inhibition of CYP2A6 by a non-toxic dose of coumarin. The OBS in combination with the MCL-5 and cH2 cell lines creates a simple, homogeneous, streamlined method for screening compounds for metabolic toxicity.

Test Substrate	cH2 cells	MCL-5 cells	Major P450's
Benzopyrene	IC50>20uM	1.6uM	1A1, 3A4, 1B1
Aflatoxin	IC50>0.2uM	0.0479uM	1A1, 3A4, 1A2
NNK	IC50>480uM	153.5uM	2A6, 2E1, 2D6
Acetaminophen*	2,900uM	1,200uM	2E1, 3A4, 1A2
PhIP	111.2uM	21.28uM	1A1
Methotrexate	0.021uM	0.023uM	Direct-Acting

1208 INDUCTION OF CYTOCHROME P450 2B6 AND 3A4 EXPRESSION BY PHENOBARBITAL AND CYCLOPHOSPHAMIDE IN CULTURED HUMAN LIVER SLICES.

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Cytochrome P450 (CYP) inducibility is a major issue in drug development, as it reflects a potential risk of drug interaction. One promising *in vitro* model to predict CYP inducibility is cultured human liver slices. The region-specificity of CYP distribution, together with drug-induced histological changes, remain yet to be defined in this model. To address this issue, we tested the effects of cyclophosphamide (CPA) and of phenobarbital (PB) on CYP2B6, 3A4 and 2C9 expression. We report that basal CYP2B6 and 3A4 expression is higher in cultured human liver slices than in hepatocyte primary cultures. In addition, slices display the same region-specificity of CYP2B6 and 3A4 expression as intact liver. In-depth histological examination showed that morphological features are preserved in human liver slices. CYP2B6 and 3A4 mRNA, apoprotein and enzyme-related activities were induced by PB and by CPA. Their immunoreactivities were also increased, while their distribution was preserved on slice tissue sections. The cytoplasm took on a ground-glass aspect characteristic of smooth endoplasmic reticulum proliferation associated with microsomal enzyme induction. CYP2B6 and 3A4 induction was variable among slices prepared from different donors, although to a lesser extent than in hepatocyte monolayers. In conclusion, cultured human liver slices are an attractive alternative to hepatocyte culture for the prediction of human CYP isoenzyme induction by xenobiotics (Supported by EU Biotechnology Program, B104-CT97-2145).

1209 INDUCTION OF CYTOCHROME P450 (CYP) FORMS IN CULTURED PRECISION-CUT HUMAN LIVER SLICES.

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In this study we have employed a panel of antipeptide antibodies to examine the induction of CYP forms in cultured human liver slices. Liver slices (diameter 10 mm, thickness around 250 μm) were prepared from samples of human liver (surplus to transplant requirements) with a Krumdieck tissue slicer and were cultured in Williams medium E containing 0.1 μM insulin, 0.1 μM dexamethasone (DEX), 2 mM L-glutamine, 50 μg/ml gentamicin and 2.5 μg/ml fungizone in a dynamic organ culture system in an atmosphere of 95%O_2/5%CO_2. Slices were cultured in control medium or medium containing either 0.05 mM β-naphthoflavone (BNF), 0.05 mM lansoprazole (LANS), 0.5 mM sodium phenobarbitone (NaPB), 0.05 mM rifampicin (RIF), 0.05 mM DEX or 0.05mM methylclofenapate (MCP) for 72hr. After culture, liver slice microsomal fractions were prepared for Western immunoblotting studies. CYP1A1 was not detectable in either control or CYP inducer treated liver slice microsomal fractions. Compared to control liver slice microsomal fractions, both BNF and LANS induced levels of CYP1A2 apoprotein. RIF induced levels of CYP2A6, CYP2B6, CYP2C19 and CYP3A4 apoproteins to 360, 1230, 305 and 440 % of control, respectively, whereas NaPB induced levels of CYP2B6 and CYP3A4 apoproteins to 980 and 255 % of control, respectively. While small effects were produced on levels of CYP2C8, CYP2C9 and CYP3A5 apoproteins, none of the CYP inducers studied had any significant effect on levels of CYP2D6, CYP2E1 and CYP4A11 apoproteins. These studies demonstrate the usefulness of liver slices for studying the induction of CYP forms in human liver. (Supported by EU BIOMED Contract No. BMH4-CT96-0254).

1210 OPTIMIZATION OF ORGAN SLICE LONGEVITY IN CULTURE TO LINK GENE EXPRESSION WITH ALTERED MORPHOLOGY.

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A procedure to maintain the morphology and function of rat liver slices up to 96 hours in culture has been developed. Slices cultured on titanium or teflon screen inserts lose their integrity at the contact point of the slice with the screen. This results in loss of tissue and normal architecture, which reduces the viability of the slice over time. Tissue quality and longevity is enhanced by organ perfusion and slicing in a preservation medium, and by culture improvements. The slices are morphologically healthy and metabolically active, synthesizing glycogen from the nutrients of the medium. The liver of Wistar rats (250-300 g) is perfused *via* the portal vein with heparinized University Wisconsin (UW) solution to cool and flush the organ of blood. The organ is cored (8 mm^3) and sliced (200 μm) in cold oxygenated UW solution using a Brendel/Vitron tissue slicer. The slices are loaded onto titanium-screen inserts covered with a mixed cellulose-ester filter, and cultured at 37°C, 85% O_2 and 5% CO_2 in a dynamic organ roller culture system in DMEM plus 5% FCS which is changed after 1 hour of incubation (T_0), and then every 24 hours. The filters maintain the viability and morphology of the slices by preventing cell necrosis and loss on contact with the screens (titanium or teflon). This was corroborated by the viability data. Slice ATP and GSH levels increased during culture until 48 hours, then stabilised. Morphological features of repair appeared after 48 hours and were characterised by the proliferation of stellate cells and neobiliary ducts. The

RNA extracted from the slices is suitable for genomic analysis. The extension of slice culture survival enhances the ability to investigate mechanisms of toxicity and to link genomics and proteomics to an altered morphology.

1211 MORPHOLOGICAL AND BIOCHEMICAL INTEGRITY OF HUMAN LIVER SLICES: EFFECTS OF OXYGEN TENSION.

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We tested the effects of low (20% O_2) and high (70% O_2) oxygen tension on the morphological and biochemical integrity of human liver slices incubated for up to 72 h in supplemented Williams' E medium in a dynamic rotating culture system. High oxygen tension was more effective than low oxygen tension for preserving morphological integrity in long-term culture (48-72 h). After 72 h of culture with 70% O_2, the lobular pattern was well preserved, and the survival of hepatocytes (approximately 80%) and other cell types was good. Immunohistochemical studies showed good preservation of the region-specific expression of CYP 2E1 and 3A4 isoenzymes for up to 72 h of incubation in 70% O_2. As compared to 20% O_2, the oxidized glutathione content and reactive oxygen species production were slightly increased in 70% O_2, suggesting that minimal oxidative stress occurred with the high oxygen tension. In conclusion, despite slight oxidative stress associated with high oxygen tension, 70% O_2 appeared more appropriate than 20% O_2 for preserving the morphological and biochemical integrity of human liver slices cultured in a dynamic organ culture system for up to 72 h. (Supported by EU Biotechnology Program, B104-CT97-2145).

1212 THE CYTOTOXICITY AND METABOLIC CHANGES INDUCED BY LEWISITE IN *IN VITRO* EXPOSED HUMAN LEUKOCYTES.

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To study the mechanism of vesication for the development of effective countermeasures, the cytotoxic and metabolic effects of the potent chemical warfare vesicant, lewisite (arsine, dichloro [2-chlorovinyl], L) were investigated in a human leukocyte model. Human leukocyte preparations were isolated by Percoll centrifugation and suspended at a final concentration of 10^6cells/ml in Tyrode's buffer with either buffer or L for various times. To determine the cytotoxicity of L at concentrations between $2x10^{-6}$ to $8x10^{-5}$ M, the cells were incubated for 1, 2, 4, 8, 12, 16, 18, and 22 hrs post-exposure before propidium iodide was added. The percent of viable cells was measured by using flow cytometry. The concentration-dependent cytotoxicity of L was detected as early as 1 hr post-exposure in the range of $1x10^{-5}$ to $6x10^{-5}$M. At the longer time intervals, cytotoxicity could be detected at lower concentrations of L. In the investigation of the effect of L on metabolism, exposures were conducted at various concentrations ranging from $6x10^{-7}$to $6x10^{-4}$ M. After exposures, the cells were harvested by centrifugation and their ATP levels were measured by the LKB-Wallac 1243-107 ATP Assay Kit in the LKB-Wallac 1250 Luminometer. Only leukocytes exposed to $6x10^{-4}$ M L demonstrated an initial decrease in ATP within 15 min after exposure. At 1 hr post-exposure, there was a concentration-dependent decrease in ATP. By 2 hrs post-exposure, the concentration-dependent decrease appears to be almost linear. The level of ATP then appears to plateau at all the concentrations of L studied from 4 to 24 hrs post-exposure. It appears that any assay designed to determine whether an antivesicant antidote directed against L is efficacious should be given during the first hr of post-exposure to block the effects on both viability and metabolism.

1213 DEVELOPMENT OF *IN VITRO* ANTIGENICITY STUDY EXPOSING TO TEST COMPOUNDS IN *IN VITRO* PRIMING CULTURE BY USING DENDRITIC CELLS (DC) INDUCED FROM BONE MARROW CELLS (BMC) IN MICE.

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In early stages of drug discovery, evaluations of toxicities are very important to reduce the attrition rate of the compounds in later stages of drug discovery and development. But, high throughput toxicity evaluation systems are not well established. In this paper, we investigated an *in vitro* antigenicity testing method by using mouse DC induced in *in vitro* culture system from BMC. BMC were isolated from mice and cultured with various cytokines (granulocyte macrophage-colony stimulating factor (GM-CSF) + interleukin-4, GM-CSF + tumor necrosis factor alpha, or GM-CSF + interleukin-6 + flt-3 ligand) for 6 days. The cell numbers were increased during the culture period, and cells having characteristics of DC (dendritic morphology, expression of DC markers; Ia, CD40, CD80 and CD86) were induced after 4-5 days of the culture. To confirm an antigen-presenting activity of the induced DC, the DC were exposed to positive reference compounds (antigens) in the culture system (a priming culture) for 1 day, and intraperitoneally injected in nontreated mice for 2-times in a 2 week interval. At 1 week after the last injection of primed DC, sera were isolated for the measurement of positive reference compound-specific antibodies, and splenocytes were isolated for an *in vitro* lymphocyte proliferation test (response to the antigens). High antibody titers were observed to bovine serum albumin (BSA), and positive antibodies were observed to dinitrobenzene sulfonic acid, sodium salt (DNBS) and trimellitic anhydride. Splenocyte proliferations were observed to BSA, DNBS and penicillin G. These results show that functional DC which have an antigen-presenting activity were induced by the culture of mouse BMC with the cytokines. These DC would be a candidate for antigen-presenting cells in *in vitro* antigenicity tests.

1214 RESPONSIVENESS OF MONKEY PLATELETS AGAINST THREE DIFFERENT AGGREGATION AGENTS.

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This experiment was designed to compare non-human primate platelet aggregation using three aggregation agents. These data were also compared to human platelet aggregation with the same agents. Twelve male and five female cynomolgus monkeys and seven male human volunteers supplied platelet specimens. Maximum platelet aggregation rates (MPAR) in monkey platelets, after the addition of Adenosine Diphosphate (ADP; 5, 10 and 20 μM), Collagen (COL; 10μ/mL) and Epinephrine (EPI; 300 μM), were evaluated by gender and compared to human data. Lipoprotein levels of monkey plasma were analyzed by electrophoresis and compared to human electrophoresis data. Both monkey and human MPAR increased dramatically after the addition of COL, and 10 or 20 μM ADP. Monkey MPAR was slightly increased after a 5μM ADP addition. Monkey MPAR only showed a minimal response to an EPI addition in comparison to the human MPAR response. There was also no gender difference in monkey platelet aggregation induced with 20μM ADP, COL or EPI. It was previously reported that the MPAR response, after EPI treatment, was significantly less in cynomolgus monkey platelets as compared to human platelets (J.Med..Primatol.15, 1986). On the other hand, the influence of lipoproteins on platelet aggregation (Arteriosclerosis 3, 1983; Hemostasis 8, 1979; Prostaglandins Leukotrienes and Essential Fatty Acids 51, 1994) was also reported in humans. In this experiment, low-density lipoprotein (LDL) levels in monkeys were about two-fold lower than in humans. Different LDL levels between both species were considered as the possible cause of low sensitivity in monkey platelets against EPI. In conclusion, monkey platelets showed similar or different responsiveness against three aggregation agents when compared to humans.

1215 HEMOLYTIC POTENTIAL OF VEHICLES USED FOR RAPID *IN VIVO* SCREENING OF SMALL MOLECULES.

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Vehicles that are administered parenterally *in vivo* may be screened *in vitro* to determine the degree of hemolysis in erythrocytes and to evaluate the effect, if any, of varying vehicle concentrations on hemolysis. This study assessed the hemolytic properties of various vehicles used for rapid *in vivo* screening of small molecules when exposed to rat blood *in vitro*. All the test articles (Tween 80, ethanol, hydroxypropyl β cyclodextrin, polyethylene glycol 400, propylene glycol, poloxamer 188, dimethyl sulfoxide, dimethyl acetamide and multiple component vehicles) were diluted to the desired concentrations in phosphate buffered saline solution. Mixtures containing equal volume of blood and each vehicle dilutions were incubated under static conditions for 30 minutes at 37 ± 1°C. The tubes were centrifuged, and the supernatant harvested were observed visually for the appearance of red color and the optical densities were read at wavelength of 545 nm. A hemolytic index for each vehicle formulation was determined by comparing the optical density determined spectrophotometrically in the test article samples to the optical density under conditions of 0% and 100% hemolysis. There was a positive correlation between plasma color and optical density in all samples. Hemolysis was not seen at the following concentrations in these vehicles: Tween 80 ≦ 0.125%, Ethanol ≦ 5%, Hydroxypropyl β cyclodextrin ≦ 1%, Polyethylene glycol ≦ 2.5%, Propylene glycol ≦ 5%, Poloxamer 188 ≦ 0.5%, Dimethyl sulfoxide ≦ 5% and Dimethyl acetamide ≦ 7.5%. All the multiple component vehicles (25% Ethanol-5% Cremophor EL, 10% DMAC- 20% Ethanol, 10% DMAC-40% PG and 20% PG-20% PEG 400) induced hemolysis in rat blood. *in vivo* animal studies and *in vitro* cytotoxicity studies to understand the toxicity profiles of these commonly used vehicles are in progress.

1216 SELECTION OF REFERENCE CHEMICALS TO VALIDATE *IN VITRO* CYTOTOXICITY ASSAYS FOR ESTIMATING *IN VIVO* STARTING DOSES FOR ACUTE ORAL TOXICITY.

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Sponsor: J. Bucher.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and NICEATM convened an international workshop in October 2000 to evaluate the validation status of *in vitro* methods for predicting acute systemic toxicity (see http://iccvam.niehs.nih.gov). As an interim step to developing and ultimately using *in vitro* cytotoxicity methods to accurately predict rodent oral LD50s for regulatory purposes, workshop participants recommended the use of *in vitro* methods to predict the *in vivo* LD50 starting dose in situations where other credible information was unavailable. It was conjectured that such an approach would further reduce the number of animals required for acute toxicity testing. In a joint effort with the USEPA, and in collaboration with the European Centre for the Validation of Alternative Methods (ECVAM), NICEATM is organizing a multi-laboratory study to validate the utility of two *in vitro* cytotoxicity tests for identifying the starting dose for *in vivo* acute toxicity tests. A major aspect of the study design was the selection of an appropriate number of test chemicals that represented 1) a wide range of toxicity, 2) the types of chemicals regulated by the various US regulatory agencies, and 3) those with human toxicity data and/or human exposure potential. Based on these criteria, a preliminary list of approximately 100 chemicals was compiled by mining several publicly available data sets. A priority list of 60 chemicals representing the 6 acute toxicity hazard classifications in the Globally Harmonized Scheme was created based on compatibility with the test systems, availability and quality of existing rodent oral LD50 data, human exposure data, commercial availability, ease of shipping, and availability of information on mechanism of action. Supported by NIEHS contract N01-ES-85424 with ILS, Inc.

1217 PRELIMINARY EVALUATION OF A CYTOTOXICITY MODEL FOR PREDICTING ACUTE ORAL TOXICITY TEST STARTING DOSES.

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For almost fifty years *in vitro* cytotoxicity methods have been studied as predictors of acute lethality *in vivo*. Recently Halle has compiled a Registry of Cytotoxicity (RC) which compares the LD50 (in mmol/kg; rat or mouse) of 347 chemicals with an average *in vitro* IC50 (in mmol/l; various cell types). A graph of the RC shows reasonably good correlation between lethality *in vivo* and cytotoxicity *in vitro*; 73 % of the points lie within a +/- log 5 interval around the regression line. This has led Spielmann et al. to propose that, at a minimum, the results of *in vitro* basal cytotoxicity tests can be used to estimate the starting dose for *in vivo* LD50 tests. It has also been suggested that new cytotoxicity methods be first qualified for use in this scheme by testing them with RC chemicals having a range of toxicities and which fit closely to the RC regression line. To qualify, the new results should have a similar slope and lie within +/- log 5 of the RC regression line. We investigated whether two commonly used cytotoxicity tests (neutral red uptake in BALB/c 3T3 [3T3] and normal human keratinocytes [NHK]) would qualify under these conditions. Eleven chemicals from the RC whose *in vivo* LD50's ranged from ~0.1 mmol/kg to ~100 mmol/kg were chosen for evaluation. All chemicals were tested under code using a well-defined standard protocol. Cytotoxicity in the treated cells relative to that of control cells was determined after 24 hr (3T3) or 48 hr (NHK) exposure. When the resulting data were overlaid on the original RC graph, all new data points lay within a +/- log 5 interval around the RC regression line. The slopes of the regression lines for the new tests were 0.506 (3T3) and 0.498 (NHK) *versus* 0.625 for the RC. R2 values were 0.985 (3T3) and 0.936 (NHK). We conclude that the proposed qualification criteria are reasonable and that both of these candidate tests have met those criteria.

1218 TOXICITY TESTS USED ONLY AS SCREENS STILL NEED CAREFUL VALIDATION.

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New toxicity tests, especially *in vitro* tests, are often used as screens; i.e. they are used to preliminarily divide a test set of chemicals into positive or negative subsets. One of two strategies can then be applied. Either 1) the negatives can be accepted as correct and the presumed positives evaluated in a second tier test, or 2) the positives can be accepted and the negatives evaluated in a second tier test. There has been a tendency to assume that validation does not have to be as stringent for a screen as it does for a stand-alone test because the screen is only a preliminary assessment. However, both approaches 1) and 2) above use screens to make some firm decisions; the first to classify a chemical as non-toxic and the second to classify the chemical as toxic. It is often thought that sensitivity (fraction of known positives correctly identified) is the most important criterion and that specificity (fraction of known negative materials correctly identified) has much less significance. It follows that sensitivity alone could be characterized by using predominately positive chemicals in a validation study. We present data to show that this reasoning can lead to major errors. In approach 1), choosing a tier I test with low specificity will result in a large number of false positive materials passing through to the more expensive or animal intensive tier two test, possibly negating the advantage of having two tiers. This is especially relevant when several mechanistically specific tests are combined into a tier I battery. In approach 2), a low specificity tier I test means that many useful chemicals may be discarded, without necessarily decreasing the prevalence of positives in the subset of negative chemicals identified by the screen. Finally we show that using predominantly positive test materials to validate a screen can result in a serious overestimation of the test's sensitivity. For any test to be useful as a screening test or as a stand-alone test, it must it must be both sensitive and specific for the endpoint of interest.

1219 THE SENSITIVITY AND SPECIFICITY OF A TOXICITY TEST ARE DEPENDENT ON THE DISTRIBUTION OF TOXIC CHEMICALS BEING EVALUATED.

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Often, the only measures of toxicity test performance provided after a validation study are the statistics sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and concordance. Sensitivity and specificity, which have been assumed to be independent of the chemical population being tested, are generally used as the primary indicators of test performance since NPV and PPV are commonly known to vary with changes in prevalence. The purpose of these studies was to test whether or not sensitivity and specificity are true constants. Monte Carlo simulations were used to generate four sets of data that fit a simple prediction model. The distribution of toxic chemicals was varied between four data sets evaluated in the study. The distribution of toxicity in two of the data sets was skewed such that either strongly or weakly toxic chemicals predominated. The toxicity of chemicals in the third data set was predominantly strongly and weakly toxic with few chemicals of intermediate toxicity. The fourth set contained chemicals predominantly of intermediate toxicity with relatively few strongly or weakly toxic chemicals. Cut-offs were set at different locations across the range of response and the performance statistics were calculated at each cutoff. The results showed that sensitivity and specificity, measured at identical cut-offs, were not constant across the data sets. We conclude that sensitivity and specificity should not be considered constant indicators of toxicity test performance and that more emphasis should be placed on the assessment of a toxicity test's positive and negative predictive value.

1220 RESULTS OF AN INTERAGENCY COORDINATING COMMITTEE ON THE VALIDATION OF ALTERNATIVE METHODS (ICCVAM) EXPERT PANEL EVALUATION OF THE VALIDATION STATUS OF THE FROG EMBRYO TERATOGENICITY ASSAY-XENOPUS (FETAX) FOR IDENTIFYING DEVELOPMENTAL TOXICANTS.

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One of the most important aspects of a toxicity assessment is the determination of the potential of a chemical to affect fetal development. Traditional *in vivo* rodent methods for evaluating developmental toxicity potential are time-, resource-, and animal-intensive. To reduce animal use and expedite evaluation, alternative *in vitro* approaches for compound screening have been developed but few have undergone rigorous review. FETAX measures embryonic development of the South African clawed frog, Xenopus laevis and is a test for organogenesis, which is highly conserved across amphibians and mammals. In this assay, eggs hatch and develop into embryos during exposure to the test substance. After 96 hr of development, individual embryos are evaluated for mortality, malformations, and growth inhibition. In 1998, the USEPA requested that ICCVAM evaluate the validation status of FETAX, its potential regulatory usefulness, and the critical research, development, and validation efforts needed to improve it. An Expert Panel Meeting (May 2000) deliberated on these issues (http://iccvam.niehs.nih.gov) and concluded that the performance and reproducibility of FETAX was not adequate for regulatory applications and recommended that: 1) the FETAX protocol be modified to address specific limitations noted by the Panel, 2) the number of endpoints be expanded to improve assay performance for identifying developmental toxicants, 3) additional,

more robust decision criteria be developed, and 4) additional validation studies be conducted. In addition, the Panel recommended that validation criteria specific for developmental toxicity, and reliable and accurate comparative databases of animal and human developmental toxicants be developed.Supported by NIEHS contract N01-ES-85424 with ILS, Inc..

1221 MONOCLONAL ANTI-PHENCYCLIDINE (PCP) IgG PRODUCES LONG-TERM PROTECTION AGAINST ADVERSE EFFECTS OF PCP.

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The purpose of this study was to determine if anti-PCP IgG protects against PCP-induced effects on locomotor activity, health and brain monoamine levels. Sprague-Dawley rats (2 groups, N=4) received 18 mg/kg/day of PCP continuously for 2 weeks *via* a subcutaneous osmotic minipump. Once PCP reached steady-state concentrations (<24 hr after implanting pump), baseline locomotor activity was determined. One group of rats then received a control saline treatment and the other group received a single dose of anti-PCP IgG, which was 0.3 times the molar equivalent amount of PCP in the body at steady-state. PCP-induced locomotor activity was determined immediately following the saline or IgG administration. The anti-PCP IgG caused a significant reduction in PCP-induced locomotor activity. Body weights were recorded for the entire 2 week experiment. Significant weight reductions were found in the rats receiving the saline. In stark contrast, anti-PCP IgG treated rats maintained their body weights and apparent good health. The rats were sacrificed 2 weeks after implanting SC pumps for determination of PCP concentrations in 7 brain regions, testis and serum. Anti-PCP IgG significantly decreased PCP concentrations in serum, testis and all brain regions, except the brainstem. Brain regions were also examined for brain monoamine levels by HPLC/EC. For these studies, a saline control group (N=4) that did not receive PCP was included. DOPAC levels were decreased in the frontal cortex of the PCP-saline group compared with the PCP-IgG group and saline control group. These results suggest that anti-PCP IgG can offer significant protective effects and improved health even in the presence of continuous high dose PCP that apparently saturates anti-PCP IgG binding capacity. (Supported by NIDA Grants DA7610 and K08 DA 339).

1222 RELATIONSHIP BETWEEN PARKINSONISM AND SERUM PROLACTIN LEVEL IN MPTP-TREATED COMMON MARMOSETS.

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The main neural disorder in ParkinsonÅfs disease is degeneration of dopaminergic neurons in the substantia nigra. The MPTP-treated marmoset is an established model for ParkinsonÅfs disease, since MPTP produces motor deficits characteristic of those seen in patients with ParkinsonÅfs disease. Prolactin secretion is under tonic inhibitory control by dopamine (prolactin release-inhibiting factor). We examined the relationship between parkinsonism and serum prolactin level using MPTP-treated common marmosets. Male and female common marmosets (1.5-3 years old) were used for this study. Animals were treated 3 to 4 times with intravenous administration of MPTP (1.5-2.5 mg/kg) under ketamine anesthesia (with a 1 or 2 week interval between administrations). Immediately after MPTP treatment, the animals were immobile, with flexed hind quarters, postural tremors, and a loss of vocalization. Approximately 6 months after MPTP-treatment, in control (non-treatment) and MPTP-treated animals, spontaneous locomotor activities were measured by recordings on electromechanical counters (photocell sensor). Behaviors were observed by video recording to evaluate parkinsonian symptoms. Serum prolactin levels were analyzed using ELISA. The mean locomotor activity in all MPTP-treated animals was decreased compared with that in control animals. All MPTP-treated animals exhibited the characteristic parkinsonian symptoms such as slow and/or uncoordinated movements, however, there was individual difference in the degree of these symptoms. In the prolactin analysis, MPTP-treated animals showed higher prolactin levels compared to the control animals. The results of this study demonstrate that intravenous administration of MPTP produces motor deficits even 6 months after MPTP-treatment due to degeneration of dopaminergic neurons. This suggests that the increase in prolactin is induced by reduction of brain dopamine levels in the MPTP-treated common marmosets.

1223 CROTONONITRILE ISOMERS HAVE A DIFFERENT PROFILE OF NEUROTOXIC EFFECTS IN THE RAT.

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IDPN (3, 3'-iminodipropionitrile) and allylnitrile have a number of common effects on the nervous and sensory systems of the rat, including degeneration of the vestibular and auditory hair cells, corneal opacification, and gliosis in the retina and olfactory bulb. Gliosis in other specific areas of the CNS has also been observed after IDPN. The vestibular toxicity of these nitriles results in a permanent behavioral syndrome. Crotononitrile (CRO), a mixture of *cis-* and *trans-* isomers, has been demonstrated to cause both this syndrome and vestibular hair cell degeneration. This study compared the effects of the two isomers of CRO, isolated to >93 % purity by fractionated distillation from the commercially available CRO mixture (approximately 60% *cis-* and 40 % *trans-*CRO). Adult male Long-Evans rats were administered for 3 days with vehicle control, *cis-*CRO (80, 100, 120 mg/kg per day) or *trans-*CRO (250 mg/kg per day) and the changes in corneal transparency and vestibular function were assessed. Surface preparations of the vestibular sensory epithelia and the organ of Corti were observed for hair cell loss by scanning electron microscopy. Concentrations in retina, olfactory bulbs, cerebellum, striatum and cingulate cortex of GFAP, a marker for reactive gliosis, were also determined. In a dose-dependent manner, *cis-*CRO increased the rating scores in behavioral tests of vestibular dysfunction, and caused corneal opacity, hair cell loss in both the vestibular epithelia and organ of Corti, and gliosis in retina, olfactory bulb and cortex but not in the cerebellum and striatum. This profile of neurotoxicity is similar to that shown by IDPN and allylnitrile. In contrast, *trans-*CRO did not cause vestibular dysfunction, hair cell loss or corneal opacity, although other neurotoxic actions, currently under investigation, were evident from the motor behavior of the treated animals. We conclude that the neurotoxic effects of nitriles depend on strict structural requirements; this suggest that they may act through their interaction with specific molecular targets.

1224 PRETREATMENT WITH L-CARNITINE PREVENTS HYPOTHERMIA INDUCED BY THE MITOCHONDRIAL INHIBITOR 3-NITROPROPIONIC ACID (3-NPA).

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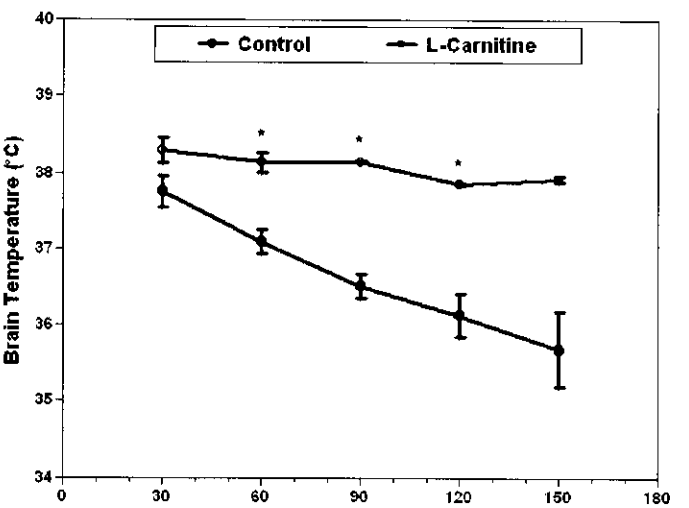
An environmental toxin, 3-NPA, can cause cellular energy deficits due to irreversible inhibition of a mitochondrial Complex II enzyme, succinate dehydrogenase (SDH). We have previously shown that exposure to 3-NPA and the resulting SDH inhibition were associated with a 2°C decrease in rectal temperature 3 hrs after dosing. While pretreatment with L-carnitine (an enhancer of free fatty acid beta oxidation) prior to 3-NPA administration, prevented the oxidative stress-associated increase in the activity of free radical-scavenging enzymes, it did not significantly prevent 3-NPA-induced SDH inhibition. Here, intracranial temperature was continuously measured near the cerebellum in adult male Sprague-Dawley rats injected with either 100 mg/kg, i.p. L-carnitine (LC) 60 min prior to 3-NPA (30 mg/kg, s.c.) or with 3-NPA alone. Rats were surgically implanted with temperature probes registering linear electrical responses to temperature change. Compared with baseline temperature, administration of 3-NPA alone was associated with a significant fall in temperature within 1 hr postinjection. Pretreatment with LC totally prevented this decrease ($p<0.05$). Data indicate that the neuroprotective effects mediated by the carnitines may result from the enhancement of energy production rather than direct or indirect interference with 3-NPA.



* indicates significant difference between L-Carnitine and Control at that time point.
Brain Temperature is average during 30 minute period

1225 NEUROTOXICITY PRODUCED BY DIBROMOACETIC ACID IN DRINKING WATER OF RATS.

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The Safe Drinking Water Act requires that EPA consider noncancer endpoints for the assessment of adverse human health effects of disinfection byproducts (DBPs). Dichloroacetic acid is a known human and animal neurotoxicant, but essentially no studies have evaluated the neurotoxic potential of the brominated analog, dibromoacetic acid (DBA). This study characterized the neurotoxicity of DBA during 6-month exposure in drinking water of rats, using a neurobehavioral test battery followed by perfusion fixation for neuropathological evaluation. Adolescent male and female Fischer 344 rats were administered DBA at 0, 0.2, 0.6, and 1.5 g/l, producing an average intake of about 0, 20, 72, and 161 mg/kg/day. In both sexes, weight gain was depressed in the high-dose group, but overall general health was not altered. Testing using a functional observational battery and motor activity took place before dosing began and at 1, 2, 4, and 6 months. DBA produced dose-related neuromuscular toxicity characterized by limb weakness, mild gait abnormalities, and hypotonia, as well as sensorimotor depression, with decreased responses to a tail pinch and click. Other signs of toxicity included decreased activity and chest clasping. The neurotoxicity did not appear progressive with continued exposure. The major neuropathological finding was degeneration of myelinated nerve fibers in spinal cord white matter (lateral and ventral areas) in the high-dose rats. In addition, small numbers of swollen, eosinophilic, and sometimes vacuolated neurites were observed in the spinal cord gray matter of mid- and high-dose rats. No treatment-related changes were seen in brain, eyes, peripheral nerves, or peripheral ganglia. Based on the neurobehavioral data, the LOEL from this study was 20 mg/kg/day (lowest dose tested). These studies demonstrate that neurotoxicity should be considered in the overall hazard evaluation of haloacetic acids. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1226 EFFECTS OF EARLY POSTNATAL ESTRADIOL AND DIETHYLSTILBESTEROL EXPOSURE ON CINCINNATI WATER MAZE PERFORMANCE OF MALE AND FEMALE RATS.

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Estrogen plays an important role in the development of the brain. Thus, exposure of developing animals to chemicals which mimic the effects of estrogen could potentially disrupt normal development leading to alteration of function including behavior. These studies were designed to determine the effect of excessive stimulation of the estrogen receptors on cognitive function using the Cincinnati water maze. Intact male and female rats were orally administered 72 µg/kg 17β-estradiol (E_2) or 10 µg/kg diethylstilbesterol (DES) from postnatal day 1 (PND1) through PND14. On PND33, behavioral testing was initiated using Path A (2 trials per day for 5 days with a 2 hour intratrial period). Following a 2 days non-testing period, rats were retested using the reverse path (Path B) in similar fashion. Neonatal exposure to E_2 significantly reduced the number of errors made by female rats in Path A. Male rats treated with E_2 also made fewer errors than controls but it was not statistically significant. The latency of the E_2 treated rats to complete Path A was lower than controls but was not statistically significant. Neonatal exposure to DES resulted in an increased number of errors and longer latency in both male and female rats in Path A but these were not statistically significant with the exception of the longer latency to complete the maze by female DES rats. Neither E_2 or DES treatment produced any differences in Path B errors or latency in either sex. Statistical comparison between the two daily trials in Path A demonstrated that both male and female E_2 treated rats made significantly fewer errors on the second trial than the first while control rats performed similarly. This was not present in the DES treated rats. This suggests that E_2 treatment during development enhances short term memory. However, since DES did not produce similar results, it can be hypothesized that this enhancement may not be mediated through the traditional E_2 receptor.

1227 NEUROTOXICITY INDUCED BY REPEATED DOSES OF 2-(DIMETHYLAMINO)ETHYL METHACRYLATE IN RATS.

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To evaluate the neurotoxicity of 2-(dimethylamino)ethyl methacrylate, groups of 6 male SD rats were administered orally with 0 (corn oil), 500, or 1, 000 mg/kg for 6 consecutive weeks. The following items were conducted on all rats: the clinical observation (twice per day); the functional assessment (once or twice per week); the histopathological examination of the central and peripheral nervous tissues. The salivation was found in the 500 and 1, 000 mg/kg groups, and the tremor of the forelimbs and the clonic convulsion in the upper half of the body was found in the 1, 000 mg/kg group. The salivation was observed immediately after dosing and appeared from the second week. The tremor was a transient sign, occurring within 30 min after dosing, and it appeared from the fifth week. The clonic convulsion was observed at the sixth week, and its trigger was the stimulation as the handling. When an attack of convulsion began, the animals crouched, thrusting out their forelimbs. Vacuolation in the white matter of the spinal cord was found in all rats of the 1, 000mg/kg group. The lesions appeared symmetrically, and they were restricted in the dorsal spinocerebellar tracts. The affected nerve fibers comprised axonal fragmentation accompanying with myelin ballooning, disruption, and phagocytosis. It was considered that clonic convulsion was due to the nerve fiber degeneration in the dorsal spinocerebellar tracts of the spinal cord.

1228 AVOIDANCE RESPONDING AND MOTOR ACTIVITY IN EPH A5 KNOCKOUT MICE.

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The Eph receptor family has been implicated in the guidance of axons and formation of topographic projection maps. For example, the Eph A5 receptor is expresed in differentiated neurons of the hippocampus and striatum and is thought to be involved in the development of the septo-hippocampal and nigrostriatal pathways. Previous neurochemical analysis revealed that Eph A5 knockout mice have higher striatal dopamine and decreased accumbal dopamine compared to wildtypes. Presently, male and female Eph A5 receptor knockout and wildtype mice were evaluated in the active avoidance responding paradigm and horizontal motor activity test. In the two-way active avoidance paradigm, mice were given 20 trials/day for 30 days; each trial consisting of a 10 sec tone CS and a 10 sec, 0.5 mA footshock US with a criterion of 18 avoidances/day. In the motor activity test, horizontal activity was recorded after 10 min in an activity monitor following baseline, saline and cocaine HCl (0.3, 1, and 3 mg/kg). In the active avoidance paradigm, male Eph A5 knockouts reached criterion by day 16, while wildtypes only reached 50% by the end of 30 days. Also, female Eph A5 knockouts reached criterion by day 22 while female wildtypes only reached 33% at the end of 30 days. Both male and female Eph A5 knockouts were less active following saline and all doses of cocaine. As both male and female Eph A5 knockout mice show enhanced avoidance responding and decreased activity after cocaine, these results may indicate a role for Eph A5 in the regulation of dopamine-mediated motor response. (Supported by NIH ES07148)

1229 CONFIRMATION OF INTER-OBSERVER RELIABILITY IN THE CONDUCT OF FUNCTIONAL OBSERVATION BATTERY ASSESSMENTS.

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The Functional Observational Battery (FOB) of is widely accepted as a sensitive tool to screen for neurotoxicity. CTBR has extensive experience performing neurotoxicity testing in accordance with GLP and stringent guidelines of the USEPA and routinely conducts of inter-observer reliability (IOR's) studies to validate technical competence in the performance of FOB tests. With the advent of the ICH S7 Guideline, these capabilities have been applied to CNS safety pharmacology studies of pharmaceuticals. The current study was a recent IOR assessment of 4 technicians and employed 3positive reference compounds: amphetamine (AM), ethanol (ET) and carbaryl (CA). Since the technicians were not drawn from the pool of previously trained staff, the IOR was preceded by a two-week training session. The IOR therefore served to validate the consistency of observations between technicians but also served to evaluate the effectiveness of the training program. The technicians were able to detect the major neurotoxic endpoints expected for each of the positive control compounds including tremors and autonomic signs with CA, ataxia and gait abnormalities with ET and bizarre stereotypical behaviors with AM. Initial variability between the gradings were no longer evident by the end of the IOR assessment. Since all technicians were considered to be scoring with similar accuracy and precision, they were approved to perform FOB assessments in rats. In conclusion, the IOR performed by our laboratory provided reliable reproducible results and confirmed the effectiveness of the training program. IOR assessments provide laboratories with a means to assess the competence of their staff and Sponsors with a means to monitor the capabilities of laboratories in the performance of such tests. In order to further reduce the risk of inter-individual or temporal variability, it is critical to perform periodic IOR assessments on technical staff assigned to such studies and whenever possible ensure the same staff carry out the FOB for all occasions on a given study.

1230 DEVELOPMENT OF A MODEL FOR CHRONIC STRESS EXPOSURE IN MICE.

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Sponsor: P. Gunasekar.

Chronic stress contributes to physical and mental illness and may interact with chemical toxins. Our previous experiments demonstrated that acute stress potentiates neurotoxicity (Lucot, 1999). Thus, we conducted controlled scientific studies of the responses to stress preparatory to studying the interactions of stress and chemical toxins. We have developed a unique model of chronic emotional stress in mice based on intermittent shaker stress. Mice are kept in their home cages fixed to a cage rack mounted on the shaker power unit. Standard pellet diet and tap water are provided ad lib. Our experimental paradigm enables the study of the neuroendocrine, cardiovascular and behavioral responses to acute as well as chronic stress. Chronic stress did not have any unfavorable effect on the health of the mice. There was 10 % of loss in body weight. However, after discontinuation of chronic stress the mean values of body weight returned to the values of non-stressed controls. Neuroendocrine and cardiovascular assessment revealed significant increases in plasma corticosterone, blood pressure and heart rate in acutely and chronically stressed mice. Chronic emotional stress did not have any effect on total open field activity or on elevated plus maze behavior. However, emotional reactivity on the elevated plus maze as measured by defecation rate increased in the chronically stressed mice 7 days after stress. Unlike the chronic stress, acute stress resulted in significant decreases in locomotor activity in the open field test. Our results show that the chronic intermittent shaker stress provides an excellent model for stress exposure and for use in testing stress/chemical interactions. Supported by DoD, contract No. 99214005.

1231 ACUTE AND REPEATED RESTRAINT STRESS HAVE LITTLE INFLUENCE ON PYRIDOSTIGMINE TOXICITY.

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Pyridostigmine bromide (PB), a quaternary, carbamate acetylcholinesterase inhibitor, has been used to protect soldiers from possible exposure to long-term inactivators of acetylcholinesterase, i.e., organophosphorus nerve agents. Some studies suggest that stress may alter BBB integrity and allow PB to enter the brain. We evaluated the effects of acute and repeated restraint stress on PB neurotoxicity. For acute restraint stress, rats were 1) restrained for 90 min and then given PB (30 mg/kg, po), 2) simultaneously restrained and given PB or 3) restrained for 3 hrs, given PB, and then restrained for another hour. In all cases, rats were evaluated for cholinergic toxicity (SLUD signs and involuntary movements) and sacrificed 1 hour after PB. Restraint stress (90 min) caused a significant increase in plasma corticosterone. Whole blood ChE was markedly inhibited (>80%) by PB whereas brain regional ChE activity (cortex, hippocampus, cerebellum) was only marginally affected (maximal 20% inhibition). PB-induced toxicity was not enhanced nor was brain ChE inhibition increased by any stress condition. BBB integrity assessed by the accumulation of horseradish peroxidase in brain regions following intracardiac tracer injection was not increased by prior stress. Rats were also exposed to daily stress (60 min) and concurrently treated with either saline or PB (3 or 10 mg/kg) for 14 days. PB-treated rats (both dosages) exhibited slight signs of cholinergic toxicity for the first 3 days of exposure after which tolerance developed. Repeated restraint, however, had little effect on functional signs of PB toxicity. Whole blood and diaphragm ChE were markedly reduced (57-84% and 74-83%, respectively) 1 hour after the last exposure but brain regional ChE activity showed minimal and inconsistent changes. The results suggest that acute and repeated restraint stress have little effect on PB toxicity or PB entry into the brain. (Supported by grant DAMD17-00-1-0070 from US Army).

1232 ADAPTIVE RESPONSE OF BLOOD-BRAIN BARRIER DISRUPTION AND INCREASED PERMEABILITY TO PYRIDOSTIGMINE BROMIDE (PB) BY REPEATED IMMOBILIZATION STRESS.

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In the present study we investigated the effects of repeated immobilization stress in male Sprague-Dawley rats on the brain penetrability of PB and its effects on the brain regional acetylcholinesterase (AChE) activity. Animals were stressed by restraining in a Plexiglas tube for 15, 30, and 60 minutes, alone or following pretreatment with 15 daily oral dose of 1.3 mg/kg PB. Brain uptake of [3H]PB (100μCi/kg, oral) was used to assess the changes in the BBB permeability. Co-exposure of PB and stress resulted in significantly increased uptake of [3H]PB (~258, 170 and164% in midbrain, cortex and cerebellum, respectively at 30 minutes of stress) and ~256% increase in brainstem at 15 minutes of stress, as compared with exposure with PB alone. However, in all the brain regions, there was a decreasing trend in the uptake following co-exposure of PB and 60 minutes of stress. PB alone caused no significant change in AChE activity in brain regions, but significant inhibition in plasma BChE activity following treatment with PB alone. Cortex and midbrain AChE activity was maximally inhibited following 30 and 15 minutes of stress alone (~77 and 52%, respectively). Co-exposure to stress and PB resulted in significant inhibition in cortex, midbrain, spinal cord, cerebellum and plasma (~36-79% of control) at 30 minutes of stress whereas maximum inhibition (~56% of control) in the brainstem occurred at 15 minutes of stress. Co-exposure to PB and stress for 60 minutes resulted in reduction in the extent of inhibition of AChE activity. These results suggest that short-term stress could disrupt the BBB permeability to PB, whereas prolonged exposure to stress results in adaptive response. Supported, in part by the US army Medical Research and Materiel Command under contract #DAMD 17-99-1-9020.

1233 VALIDATION OF A CNS SAFETY PHARMACOLOGY BATTERY IN THE MOUSE.

S. Mason, G. Washer and C. Banks. *Toxicology, CTBR, Senneville, PQ, Canada.*
Sponsor: G. Lulham.

The ICH S7 Safety Pharmacology guidelines stipulate a core battery of tests that are required for assessment of effects on the central nervous system (CNS). In addition, follow-up studies may be required on a case-by-case basis for additional information. At CTBR, a GLP-compliant series of CNS studies (functional observational battery, locomotor activity, body temperature) have historically been performed in the albino rat. For greater flexibility, both core battery and extended follow-up studies were validated using the albino mouse. Initial studies comprised a modified Irwin Screen and evaluation of spontaneous locomotor activity and body temperature. Follow-up studies included assessment of forced locomotor activity, auditory startle response, barbiturate sleep time, acetic acid writhing and anti- and pro-convulsant assays with both chemical and electrical induction of convulsions. A range of common positive control compounds with well-documented effects in these studies were used to demonstrate the effectiveness and acceptability of the assays. 6 week old male Swiss Crl:CD-1®(ICR)BR mice were used in all tests. Positive control compounds were formulated in deionized water and were administered as a single oral gavage dose or intraperitoneal injection. Dose levels were based on published literature. Animals administered sedative or stimulant drugs and assessed for general behavioral responses, both spontaneous and forced motor activity or startle response all exhibited characteristic reactions. Extensions were seen for anesthesia time following co-administration of barbiturates and benzodiazepine or phenothiazine drugs, a reduction was seen in the number of writhes induced by acetic acid following both opioid and non-opioid analgesic administration, and escalation or diminution of the convulsive threshold was observed for both chemical and electrical induction of convulsions following alkaloid or chlordiazepoxide administration. All studies therefore met their pre-defined acceptance criteria and both the core battery and follow-up studies are considered validated for use at this laboratory.

1234 A FUNCTIONAL OBSERVATIONAL BATTERY (FOB) FOR USE IN DOG TOXICITY STUDIES: DEVELOPMENT AND VALIDATION.

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The most commonly used nonrodent species in regulatory systemic toxicity and safety assessment studies has been and remains the dog, with the beagle being the standard breed employed. While a standardized FOB for use in rats (based on Gad, 1982) has been incorporated into rodent studies since the late 1980's, and some work has been previously conducted towards developing a similar screen in the dog (Haggerty, 1991), progress has been limited. Given both the differential metabolism and sensitivity of the dog compared to rodents, and the extreme desirability of having as complete a set of toxicity and/or functionality measures in the same species (to simplify and improve the accuracy of dose/response metrics), the need for such a validated methodology is clear. Study data from prior work establishes the susceptibility of dogs to a wide variety of neurotoxic agents, including 6-ANA, methanol, lasalocid, metronidazole, acrylamide, clinoquinol, organo tins, and mercury. Additionally, the dog is likewise well established as a sensitive model for a wide range of peripheral and central nervous system active pharmacologic agents, as required by recent regulatory requirements for safety pharmacology evaluations. A robust and yet sensitive noninvasive screening methodology for detecting and pro-

viding initial quantitation and characterization of such direct and indirect neurotoxic and neuropharmacologic effects has been developed. Additionally, an analysis and interpretation component which allows differentiation of neurotoxic from neuropharmacologic activities, has been developed and validated. Comparative species and strain differences in sensitivity to neuroactive agents are alos discussed, as well as means for integrating these measures into existing standard designs.

1235 CONTINUED USE OF A BEHAVIORAL TEST BATTERY TO ASSESS RHESUS MONKEY OFFSPRING EXPOSED TO COCAINE *IN-UTERO.*

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In order to explore possible long-term effects of gestational cocaine (COC) exposure, pregnant rhesus monkeys were treated with COC chronically from about 1 month (mo) of gestation until term (either 0 (N=3), 0.3 (N=3), 1.0 (N=3), or escalating doses up to 8.5 (N=3) mg/kg (i.m.), 3 times/day, 5 days/week). Begining at 6 mo of age, the behavior of offspring (N=12) was monitored using an operant test battery with 4 food-reinforced tasks designed to model aspects of motivation, learning, color and position discrimination, short-term memory and attention. Between 6 and 18 mo of age, the acquisition of operant behaviors by juvenile offspring was not differentially affected by gestational COC exposure. Possible long-term effects of gestational COC exposure were examined further by measuring the sensitivity of acquired operant behaviors in young adult offspring to acute administration of SCH23390 (SCH: 0, 0.010, 0.030, 0.056, 0.100 or 0.175 mg/kg (i.v.)), quinpirol (QN: 0, 0.10, 0.30, 0.56, 0.75 or 1.00 mg/kg (i.m.)), or spiperone (SP: 0, 0.001, 0.003, 0.010 or 0.030 mg/kg (i.m.)). When assessed at 54 (SCH), 62 (QN), or 67 (SP) mo of age, the operant behavioral responses of offspring to acute challenges were not differentially affected by gestational COC exposure. It was concluded that gestational COC exposure had no significant effect on the subsequent acquisition of complex behaviors by offspring, or the sensitivity of those behaviors to pharmacological challenge with SCH, QN or SP. Supported by NIDA IAG #224-89-003

1236 NEUROLOGICAL ABNORMALITY IN WORKERS OF 1-BROMOPROPANE FACTORY.

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It is been demonstrated that 1-bromopropane (1-BP), an alternative to ozone-depleting solvents, produces neurotoxicity and reproductive toxicity in rats. This study investigates the neurological effects of 1-BP in workers exposed to low concentrations of 1-BP. The survey examined 25 female workers at a 1-BP factory, 37.8Å}7.1 (Mean Å} S.D.) years old, and 27 female workers from a beer factory 35.8Å}5.8 years as controls. Individual time weighted average exposure levels of the workers in the 1-BP factory were estimated using individual passive sampler tubes to be 0.32-49.19 (median1.06) ppm. Neurobehavioral and electrophysiological assessments of nerve function were performed in both groups. Vibration sense deficits were detected in the feet of 17 exposed workers but not in any of the control subjects. Distal lantencies of the exposed workers were significantly longer than the controls, but the motor nerve conduction velocity of the tibial nerve did not show significant differences. Conduction velocities of the sural nerve and the F-wave of the tibial nerve were significantly decreased in the exposed workers relative to controls. Digit Symbol, Benton Visual Retention and Pursuit Aiming tests produced inferior values for the exposed group. In the Profile of Mood States testing, the exposed group exhibited lower scores than the control in Tension, Depression, Anger, Fatigue and Confusion. These findings suggest that exposure to 1-BP at levels below or around 50 ppm in the occupational setting may affect peripheral and central nervous system function.

1237 TOXICOKINETIC-TOXICODYNAMIC RELATIONSHIPS IN HUMAN MEPROBAMATE POISONINGS.

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The value of PK-PD relationships in clinical pharmacology is now well recognized. However, the potential interest of Toxicodynamic-Toxicokinetic (TK-TD) relationships in medical toxicology has been poorly investigated. The aim of this study was to correlate the depth of coma with plasma meprobamate (MB) concentrations. Materials and Methods : Plasma MB concentrations were measured using a colorimetric assay. The depth of coma was assessed using the Glasgow coma scale (GCS). Non-linear regression was used for modeling TK-TD relationships. Results : TK-TD relationships were studied in 7 acute MB poisonings. Three patients were previously treated with MB. Previous treatment was unknown in 2 patients. Mixed drug poisoning was noted in the 7 patients. The mean GCS at the time of hospital admission was 4 + 1, the mean plasma MB concentration was 1054 +/- 318 µmol/l. The mean plasma MB concentration associated with a GCS of 3 was 714.6 +/- 277.7 µmol/L (95% CIÜ: 546.8-882.4). The TK-TD relationship was well fitted with the sigmoidal Emax model. In the 7 patients, the mean Hill coefficient (m+/- SD) was 6.9 +/- 4.0 (95% CIÜ: 3.2-10.7), the mean C50 was 487.9 + 318.8 µmol/L (95% CI: 193.0-782.7). Two patients exhibited tolerance to the sedative effect of MB. In the 5 non-tolerant patients, the mean Hill coefficient (m+/-SD) was 8.0 + 4.4 (95% CIÜ: 2.5-13.4), the mean C50 was 315.8 +/- 148.9 µmol/L (95% CIÜ: 130.9-500.7). Discussion: A maximal toxic effect (GCS of 3) was associated with a wide range of plasma MB concentrations. During the course of MB poisoning, the relationship between the depth of coma and the corresponding plasma concentrations is of a sigmoidal shape. The high value of the Hill coefficient in non-tolerant patients showed that a small decrease in plasma MB concentrations near the C50 was associated with a dramatic improvement in their level of consciousness. In non-tolerant patients, the mean C50 was close to the upper limit of the therapeutic plasma concentration of MB given by our toxicological laboratory (< 200 µmol/l).

1238 EFFETS OF PEAK CONCENTRATIONS ON THE NEUROTOXICITY OF STYRENE IN VOLUNTEERS.

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The manufacture of fibreglass reinforced plastic products may give rise to substantial peak exposures to styrene. Such exposure pattern needs further consideration in terms of styrene neurotoxicity. The aim of this study was to evaluate the neurotoxic effects of short-term peak exposures in volunteers, at levels respecting the Quebec occupational exposure limits (8-h-TWA of 213 mg/m^3 and 15-minutes average of 426 mg/m^3). The volunteers had not been previously exposed to styrene and they had no documented exposure to known neurotoxicants during the study. Forty volunteers were exposed to five exposure scenarios during 6 hours : a) stable exposure to 106 mg/m^3, b) variable exposure with a mean concentration of 106 mg/m^3 with four 15-minutes peaks mounting up to 213 mg/m^3, c) stable exposure to 213 mg/m^3, d) variable exposure with a mean concentration of 213 mg/m^3 and 4 peaks of 426 mg/m^3 and e) two stable exposures to 5 mg/m^3 (control). Before and after each exposure scenario, volunteers were submitted to a battery of sensory tests (visual and olfactory), neuropsychological tests (reaction time, attention, memory, psychomotricity), and self-evaluation questionnaires (mood and symptoms) in a test-retest design. The results show that the different exposure scenarios negatively influenced neither the performance to any test nor subjective signs and symptoms. Thus, the short-term exposure, below the Quebec occupational exposure limits does not induce neurotoxic effects evaluated using a battery of internationally recognized neurotoxic tests. Due caution must be exercised in extrapolation the current results to occupational exposure since only acute exposures were used and volunteers were at rest during exposure, which exposed them to lower doses than those experienced by physically active workers. This project was supported by IRSST, Quebec, Canada.

1239 BEHAVIOURAL EFFECTS OF PEAK EXPOSURE TO TOLUENE IN THE RAT.

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Occupational exposure limits of solvents are usually based on average exposure levels during the work shift. However, recent insights recognize that the effects of inhalatory exposure to solvents may not only depend on the total dose administered but also on the exposure scenario, e.g. exposure to fluctuating vs constant concentrations. In two inhalatory exposure studies, rats were exposed to constant toluene concentrations of 1333 or 2667 ppm or to fluctuating concentrations (cycles of 1.5 h each with peaks of 8000 ppm for 15 min or 30 min) for 7.5 hours. Rats received an overall similar total dose of 10000 or 20000 ppm-hours, respectively. Visual discrimination performance was tested prior to exposure, after the 7.5-h exposure period and one day post-exposure. The different exposure scenarios induced different changes in measures of response speed, stimulus control and disinhibition of responding, compared to air-exposed rats. Slowing of response was most pronounced when measured sometime after the end of a peak exposure, the effects on stimulus control and disinhibition of responding were most pronounced when measured immediately after a peak exposure or after a period of exposure to a constant concentration. In one study, spontaneous motor activity was measured continuously

for 72 h, from one day prior to one day after the exposure-day. Different effects were observed on motor activity depending on the exposure scenario. During peak exposure, increased activity was observed during exposure, with an even larger increase immediately after the end of a peak exposure. Behavioural effects appeared to be dependent on the exposure scenario. Comparison of behavioural data with toxicokinetic data showed that the concentration of toluene in the brain is not the only determinant of behavioural effects.

1240 PHYSIOLOGICALLY-BASED TOXICOKINETIC MODELLING OF PEAK EXPOSURE *VERSUS* CONSTANT EXPOSURE TO TOLUENE IN THE RAT.

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The health risks of inhalatory exposure to volatile organic solvents may not only depend on the total external dose, but also on the pattern of exposure. It has been suggested that exposure to regularly occurring peak concentrations (peak exposure) may have a stronger impact on the brain than constant exposure. Physiologically based toxicokinetic (PBTK) modelling may be a useful tool to assess the internal dose at target sites (e.g. the brain) as a result of various peak exposure scenario's compared to constant exposure. In two inhalatory exposure studies, toluene was selected as a model compound to evaluate whether PBTK-modelling can accurately predict the internal concentrations in the rat for different patterns of inhalatory exposure. The PBTK-model was constructed with parameters from literature. In order to validate the model, experimentally obtained data from groups of rats exposed to constant toluene concentrations of 1333 or 2667 ppm or to fluctuating concentrations (cycles of 1.5 h each with peaks of 8000 ppm for 15 min or 30 min) for 7.5 hours were used. Rats received an overall similar total dose of 10000 or 20000 ppm-hours, respectively. Toluene concentrations were determined in blood and brain at a number of time points during and after exposure. The experimental results showed relatively small differences in the kinetics of toluene between constant and peak exposure scenarios. The exposure scenario was found to have the most pronounced effect on the maximum concentrations of toluene in blood and brain. The predictions of the PBTK model corresponded well with the experimentally obtained data. The difference in the predicted area under the tissue concentration *versus* time curve (AUC) of toluene in blood and brain up to 24 hours after the start of exposure was very small between both types of exposure.

1241 ADOLESCENT NICOTINE ADMINISTRATION: EFFECTS ON SEROTONIN (5HT) RECEPTOR SYSTEMS.

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Adolescent smoking is highly associated with the subsequent development of depression, a condition related to disruption of 5HT function. We administered nicotine to adolescent rats on postnatal days (PN) 30-47, using an infusion paradigm that reproduces the plasma nicotine levels found in smokers, and examined 5HT receptors and related cell signaling during nicotine administration (PN45) and in the withdrawal period, concentrating on brain regions possessing major 5HT terminal projections. Adolescent nicotine treatment decreased 5HT2 receptor binding in the cerebral cortex, with a preferential effect on females. Reductions also were seen in the hippocampus across both genders, appearing during the withdrawal period and persisting for at least one month after discontinuing nicotine. In contrast, there were no significant changes in 5HT1A receptors. We also detected major changes in cell signaling pathways, exemplified by adenylyl cyclase (AC). Adolescent nicotine elicited significant induction of hippocampal AC, as assessed with the direct AC stimulant, forskolin. In addition, the ability of 5HT1A receptors to inhibit AC was enhanced during the withdrawal period, again with the effects restricted to females. Our results indicate that adolescent nicotine exposure influences the expression and function of postsynaptic 5HT receptors and their associated signal transduction cascades in brain regions associated with affective disorders, learning and memory. The gender selectivity seen here is consistent with prior work with markers of cell damage, and also with the higher incidence of depression in human females. Further studies of 5HT systems may provide a mechanistic link between adolescent nicotine exposure and affective disorders. (Supported by USPHS DA14247)

1242 EFFECT OF EPISODIC WEEKLY NICOTINE ADMINISTRATION ON REPEATED ACQUISITION IN RATS.

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Prior research (MacPhail et al., 2000) showed both tolerance and sensitization to nicotine's effects on the motor activity of rats when dosing occurred weekly. This experiment determined the generality of this finding to conditioned behavior. Adult male Long-Evans rats (N=18) were reinforced for emitting three-response sequences in standard operant chambers. Each daily session consisted of a multiple schedule with a new sequence to be learned (i.e., repeated acquisition) and a fixed sequence that was invariant (i.e., a performance control). Following extended training, all rats received a single dose (0.6 mg/kg) of nicotine, s.c., 5-min prior to testing. Rats were then divided, based on nicotine responses, into two groups that received either two additional injections of nicotine or saline vehicle at weekly intervals. All rats then received nicotine in the fourth week. Nicotine initially decreased response accuracy and reinforcement rate regardless of the sequence (fixed or varying). Tolerance developed to the effects of nicotine in rats dosed weekly. Substantial tolerance was also obtained in rats receiving nicotine three weeks after the first dose. No differences were obtained between the two groups during sessions when nicotine was not administered. These results suggest long-term behavioral changes in nicotine sensitivity following episodic acute nicotine administration. This abstract does not necessarily reflect USEPA policy.

1243 EFFECT OF WEIGHT MAINTENANCE AND ACTIVITY ON TISSUE LEVELS OF TRICHLOROETHYLENE IN MALE LONG-EVANS RATS.

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Trichloroethylene (TCE) is a common neurotoxic pollutant, contaminating air, water and food. Assessment of its risk to health will be aided by an exposure-dose-response model linking exposure to acute neurotoxicity. One test method for neurotoxicity being used to evaluate TCE neurotoxicity requires the use of weight-maintained animals actively working for rodent chow. Thus, part of our exposure-dose-response modeling effort is development of a physiologically-based pharmacokinetic model for TCE in weight-maintained rats. For this purpose, TCE concentrations were measured in the blood, brain, liver and fat of rats exposed by inhalation to 200 or 2000 ppm TCE for either 5, 20 or 60 min and rats exposed to TCE for 60 min and then to clean air for 60 min. Measurements were made in: weight-maintained (~350 g) rats actively performing a signal detection task during inhalation exposure to TCE; weight-maintained (~350 gm) rats exposed to TCE in the absence of task performance (sedentary); and, *ad libitum*-fed rats (~weight range, 350-450 gm) that were sedentary during exposure to TCE. The observed TCE tissue concentrations were not proportional to the external exposure concentrations as a 10-fold increase in external TCE concentration (200 to 2000 ppm) resulted in greater than 10-fold increases in tissue TCE levels. As expected, due to its lipophilic nature, the highest concentrations of TCE were observed in fat. At 200 and 2000 ppm TCE, the concentrations of TCE in blood, brain, liver and fat were higher in the active, weight-maintained rats than in either the sedentary, weight-maintained rats or the sedentary, *ad libitum*-fed rats. Thus, activity status (active vs sedentary) appeared to be a more important determinant of the pharmacokinetic behavior of TCE than weight status (*ad libitum*-fed vs weight-maintained). (This abstract may not reflect EPA policy.)

1244 EXPOSURE-DOSE-RESPONSE MODELING OF THE NEUROTOXIC EFFECTS OF ORGANIC SOLVENTS.

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Risk assessments based on exposure to volatile organic compounds (VOCs) are hampered by the complexities of exposure scenarios, a lack of data regarding the mode of action of the VOCs, and uncertainties about extrapolating from animal data to human health risk. We are developing an approach to rationalize these assessments by (1) linking airborne exposure to internal dose *via* dosimetry modeling in rats; (2) investigating interactions of solvents with receptor systems in the CNS; and (3) comparing the relative sensitivities of rats and humans to the acute effects of VOCs. Physiologically-based pharmacokinetic (PBPK) models are being used to estimate the concentrations of VOCs in blood and brain of rats using a variety of exposure scenarios. Estimates of internal dose (concentrations of trichloroethylene or toluene in blood and brain) accurately predicted the effects of these VOCs on signal detection behavior and visual evoked potentials (VEPs). Mode of action studies are underway to identify critical neurochemical pathway(s) mediating the effects of toluene on signal detection behavior and VEPs, and to test the feasibility of *in vitro* tests of the acute neurotoxicity of VOCs. Parallel tests of signal detection in rats and humans inhaling toluene may yield a quantitative estimate of the relative sensitivities of the species, to facilitate extrapolation of toxicity in rats to the risk of adverse effects on human health. (This abstract does not necessarily reflect EPA policy.)

1245 A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR TOLUENE IN THE LONG-EVANS RAT: BODY COMPOSITION AND PHYSICAL ACTIVITY.

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A physiologically-based pharmacokinetic (PBPK) model for inhaled toluene was developed for Long-Evans rats as a component of an exposure-dose-response (EDR) model for volatile organic compounds. The PBPK model was needed to link airborne toluene exposure to its concentration in blood and brain, the target organ for its neurotoxicity. The acute neurotoxicity of toluene was quantified in rats as impaired detection of visual signals and reduced visual evoked potentials (VEPs). The model required evaluation under test conditions for both of these endpoints, i.e., signal detection behavior is measured in weight-maintained, physically-active rats, whereas VEPs are measured in rats fed ad libitum and restrained during testing. Blood and brains were obtained from 64 sedentary rats, either weight-maintained (350 g) or fed ad libitum (400 - 500 g), after inhaling toluene at either 200 or 2000 ppm for 5, 20, or 60 min, or 60 min after a 60-min exposure to toluene (n = 4/gp). Weight maintenance had little effect on either predicted or observed concentrations of toluene. Effects of toluene on signal detection were not readily predicted by parameters of exposure, but were accurately predicted by concentrations of toluene in brain and blood estimated by the model. In contrast to the lack of effect of weight status, higher values of cardiac output and alveolar ventilation, expected as a result of physical activity, greatly increased the tissue concentrations of toluene predicted by the model. We are currently evaluating heart rate, obtained by telemetry from rats performing the signal detection task, as an index of activity-induced elevations of cardiac output and ventilation rate in the PBPK model. Adjusting these parameters for physical activity will improve the accuracy of the estimated concentrations of toluene necessary to impair signal detection. (This abstract does not necessarily reflect EPA policy.)

1246 EFFECTS OF TERBUTALINE (TER) ON BETA-ADRENOCEPTOR (BAR) SIGNALING IN THE DEVELOPING RAT: IMPLICATIONS FOR ADVERSE EFFECTS OF TOCOLYTIC THERAPY.

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Preterm labor is commonly treated with B2AR agonists, like TER, that arrest uterine contractions, but that also enter the fetus. Ordinarily, tissues are protected from excessive BAR stimulation by desensitization and downregulation. The current study was undertaken to determine if these mechanisms also protect the developing fetus. We modeled late 2nd-3rd trimester human exposure by administering TER (10 mg/kg s.c.) to rats on postnatal days (PN) 2-5, a period prior to the development of peripheral sympathetic function, or on PN11-14, during the onset of sympathetic function. TER downregulated cardiac and hepatic BARs at both ages, with a greater effect in the liver. Despite the reduction in cardiac BARs, there was no desensitization of cell signaling mediated through adenylyl cyclase (AC). Using stimulants that operate at different loci in the AC cascade, we found that TER treatment on PN 2-5 did not desensitize any of the signaling proteins; treatment on PN11-14 actually increased activity of the G-proteins and AC itself, distal to the BAR. Similarly, in the liver, heterologous sensitization of AC signaling offset nearly all the BAR downregulation, so that BAR-mediated cAMP generation was maintained. Our results indicate that the developing organism does not display agonist-induced desensitization of the BAR/AC signaling pathway and can actually show sensitization in response to TER treatment. In light of the critical role played by cAMP in cell replication and differentiation, these unique responses may contribute to cardiac anomalies and alterations in glucose handling noted in infants whose mothers received tocolytic therapy. Finally, numerous environmental and hormonal factors converge on the generation of cAMP, so that sensitization of the AC pathway by tocolytics may render these individuals more vulnerable to developmental disruptors. (Supported by USPHS HD09713)

1247 ENHANCED SKIN CARCINOGENICITY IN PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR β-NULL MICE.

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The peroxisome proliferator activated-receptor-β (PPARβ) shows high levels of expression in the skin, although the role of this receptor in skin function is uncertain. Interestingly, the epidermal hyperplastic response induced by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) is greatly increased in PPARβ-null mice. This suggests that PPARβ functions to attenuate epidermal cell proliferation in response to TPA. In this study, PPARβ-null and wild-type mice were subjected to the two-stage chemical carcinogenesis bioassay to elucidate a functional role of PPARβ in skin cancer. PPARβ-null mice were more sensitive to TPA than wild-type controls as evidenced by differences in skin morphology after two-three topical applications of TPA. This phenotype continued for the duration of the six month experiment. The formation of papillomas in PPARβ-null mice was greatly accelerated compared to wild-type mice and the percentage of PPARβ-null mice that developed papillomas was significantly greater than wild-type controls. After 24 weeks of TPA application, the average number and size of papillomas in PPARβ-null mice was greater than in wild-type controls. These results suggest that PPARβ attenuates cell proliferation. In the absence of a functional PPARβ, skin tumor formation and growth are greatly enhanced in this two-stage chemical carcinogenesis model, which suggests that activation of this receptor could inhibit skin cancer. Additionally, these data suggests that humans with a polymorphism or mutation in PPARβ gene may be at increased risk for developing skin cancer. (Supported by RO1 CA89607, J.M.P)

1248 MOLECULAR AND BIOCHEMICAL ALTERATIONS IN PEROXISOME PROLIFERATOR- ACTIVATED RECEPTOR-β (PPARβ) NULL MOUSE SKIN IN RESPONSE TO 12-O-TETRADECANOYLPHORBOL-13-ACETATE (TPA).

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The biological roles of PPARβ remain largely unknown. The use of the PPARβ-null mouse model should greatly advance our understanding of how this receptor functions in target tissues. Recent evidence suggests a role for PPARβ in epithelial cells. In the absence of PPARβ, epidermal hyperplasia is greatly enhanced in response to TPA suggesting that PPARβ may function to attenuate cell proliferation. Potential PPARβ target genes that could regulate the cell cycle have not been identified. Using microarray analysis, several genes were identified that are likely regulated by PPARβ. Northern Blot analysis confirmed that expression of these genes in response to TPA is mediated by PPARβ. The specific role of these target genes in epithelial cell cycle control is currently under investigation. In addition to molecular alterations, biochemical changes in intracellular phosphorylation events were also examined since this type of regulation is a common mechanism found in replicating cells. Significant differences in phosphorylated proteins were found between wild-type and PPARβ-null mouse skin in response to TPA. Specifically, cytosolic levels of phosphorylated mitogen-activated protein kinase kinase (MAPKK) and several protein kinase C (PKC) isoforms were approximately 2-fold higher in null mouse skin treated with TPA compared to wild-type controls. Additionally, western blot analysis revealed that the translocation of PKC from the cytosol to the particulate fraction is markedly different between TPA-treated PPARβ-null and wild-type skin. Combined, these results suggest that PPARβ regulates epidermal target gene expression that ultimately leads to modulation of phosphorylation events, which in turn regulate cell cycle progression. (Supported by RO1 CA89607, J.M.P.)

1249 DIFFERENTIAL EFFECTS OF 3'-METHOXY-4'-NITROFLAVONE (3'M4'NF) ON INDUCTION OF LUCIFERASE AND CYP1A1 IN RECOMBINANT MOUSE HEPATOMA CELLS.

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Aryl hydrocarbon receptor (AhR) is a transcription factor belonging to the bHLH-PAS gene superfamily. Ligand-free receptor resides in cytosol as a complex associated with several other proteins. Upon ligand activation, the receptor undergoes a series of transformation process that involve translocation to the nucleus, association with AhR translocation partner (Arnt), and interaction with dioxin responsive element (DRE), leading to the enhancement of gene expression such as *CYP1A1*. Cell lines stably transfected with AhR-responsive, mouse *CYP1A1* DRE-driven luciferase (*2DLuc*) are sensitive bioassay systems for the study of AhR signaling mechanisms and determining the presence of dioxin-like environmental contaminants. Although studies have shown that TCDD induces *2DLuc* and *CYP1A1* in a similar pattern, there is little information about the induction of these two genes by structurally different AhR ligands. It is possible that dissimilar ligands may elicit a different AhR conformation, which may lead to distinct expression profiles between *2DLuc* and *CYP1A1* (or other genes). To test the hypothesis, the recombinant cells were treated with 3'M4'NF. The induction of *CYP1A1* or *2DLuc* was examined by three different endpoints: mRNA transcription, protein expression and enzyme activity. The studies demonstrated that 3'M4'NF produces a differential effect on the induction of reporter gene and *CYP1A1*. 3'M4'NF has no or little activity in enhancing the expression of *2DLuc* by itself but is able to antagonize TCDD-induced reporter gene induction completely, an indicative of 'pure' antagonist. In contrast, it promoted the *CYP1A1* expression in a dose-dependent manner, an indicative of partial agonist. The results presented herein provide us further insights and qualifications for the use of reporter genes to study the AhR signaling pathway. (Supported by NIH grant ES09702 and Center grant ES01247)

1250 2D GEL ELECTROPHORESIS, MUTATION ANALYSIS, AND SEQUENCE/STRUCTURE-BASED PHOSPHORYLATION PREDICTION OF ARYL HYDROCARBON RECEPTOR (AhR) TYROSINE RESIDUES SUGGEST PHOSPHORYLATION AT TYROSINE 9.

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The AhR is a ligand-activated member of the bHLH transcription factor family. Xenobiotics such as TCDD bind to the AhR to mediate pleiotropic toxicities. For the AhR, a phosphoprotein with demonstrated charge heterogeneity, DNA binding is a critical step necessary to modulate target genes that mediate TCDD toxicity. We previously reported that treatment of a purified AhR/Arnt complex with tyrosine (Y) phosphatases or mutation of AhR Y9 to phenylalanine (AhRY9F) abrogates DNA binding *in vitro*. We hypothesized that utilization of computational analysis together with 2D gel electrophoresis (2-DE) of AhR Y mutants would correlate AhR charge heterogeneity with predicted Y phosphorylation and indicate a possible role for AhR Y9 phosphorylation in DNA binding. An N-terminal truncated mutant (AhR1-399) was shown to retain DNA binding potential *in vitro*. Point mutations of each of the twelve N-terminal Y residues indicated that only the Y9 mutant abrogates DNA binding, suggesting a specific functional role for this conserved amino acid. Computational sequence and structure-based prediction analysis indicated that Y9 also maintains conserved phosphorylation potential across all species sequenced. To corroborate this potential, *in vitro* translated AhR WT and point mutants were analyzed by 2-DE. These data indicate more basic isoforms for AhRY9F and other sites with a high predicted phosphorylation potential (AhRY372F) while sites with low predicted phosphorylation potential (AhRY365F) have isoforms similar to AhR WT. These data suggest consistency between charge heterogeneity and the predicted phosphorylation potential of AhR Y residues and putative phosphorylation at AhR Y9. This, along with previous data, strongly suggests that Y9 phosphorylation plays a role in AhR DNA binding. (Supported by NIH Grant ES02515, Training Grant ES07026, and Center Grant ES01247.)

1251 COMPREHENSIVE ANALYSIS OF GENE EXPRESSION IN HEPATOMA CELLS EXPOSED TO THE PEROXISOME PROLIFERATOR WY, 14, 643: EVIDENCE OF AN AUTOREGULATORY PATHWAY.

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor family. PPARs are activated by a large group of chemicals that include industrial pollutants which are called peroxisome proliferators (PPs). PPs are believed to cause cancer in rodents by altering gene expression and affecting the phenotype of the target cell. Examination of gene expression microarrays from rat hepatoma cells treated with the PP Wy14, 643 showed the expected increase in mRNA for fatty acid catabolism genes including acyl-CoA oxidase, fatty acid binding protein and cytochrome P450 4A1. A wide variety of lipid metabolism, cell cycle and growth regulatory genes were also observed to be PP-responsive. The expression microarray experiments also revealed a variety of protein kinases, phosphatases, and signaling molecules as being PP-responsive. Several of these proteins have been shown to affect PPARα activity as well as being key players in the cell cycle. For example, members of the MAPK, JAK/STAT and PKA pathways were affected by PPs at the mRNA, protein and activity level. Inhibition of these kinase cascades results in an alteration in PPARα activity. In addition, we tested the hypothesis that PP's ability to regulate kinase cascades may affect the phosphorylation of important cell cycle regulators. Of particular interest is Retinoblastoma (Rb). Following treatment with PPs, an increase in Rb phosphorylation at serines 807 and 811 is observed. We are studying the mechanism by which this important protein is being affected by PPs. The data from this study can also be used to speculate on other kinase pathways that affect this nuclear receptor and also heighten awareness of the importance of cross-talk between PPAR and growth factor signaling. (Funded by NIH ES07799 and DK49009).

1252 EFFECTS OF CYTOSOLIC CONVERSION OF ESTRONE TO ESTRADIOL ON RAINBOW TROUT ER BINDING AFFINITY.

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The relative binding affinity (RBA) for estrone (E1) to the rainbow trout (*Oncorhynchus mykiss*) estrogen receptor (rtER) was measured as part of a larger effort to determine chemical structural features predictive of chemical estrogenicity in fish. Estrone RBA was found to vary considerably (14 to 68%) among cytosolic preparation more than expected due to experimental variability. The cytosolic concentrations of E1 after 20h incubation at 4°C (binding assay conditions) was subsequently found to vary from 32 to 85% of the original E1 concentration, raising concerns over possible differences in hydroxysteroid dehydrogenase levels in individual fish. In mammals, 17β-hydroxysteroid dehydrogenases (HSD) are responsible for the interconversion of estrogens and androgens to and from active and inactive forms. HSD 1 has been identified as the primary enzyme responsible for the conversion of E1 to 17β-estradiol (E2). Mammalian HSD 1, localized in ovary and placenta, is present at low levels in mammalian livers. Little is known about HSD activity in fish but likely a HSD 1-like enzyme in liver cytosol preparations converts E1 to E2. Depending on the cytosol used, 15 to 68% of the initial E1 was converted to E2. No conversion was detected in water or cytosol buffer under the same conditions. A positive correlation was noted between increasing cytosolic E2 levels and RBA presumably attributable to the interaction of E2 with the rtER. A similar variation in E1 to E2 conversion (48 to 71% E2) was observed when lake trout (*Salvelinus namaycush*) liver cytosols were exposed under the same conditions. Therefore it appears that measurable HSD-like activity in fish cytosolic preparations can readily alter steroid concentrations, and alter apparent RBA measurements, and that the RBA of rtER for E1 is closer to 14% than 68%. *This abstract does not necessarily reflect EPA policy*

1253 CHARACTERIZATION OF ARYL HYDROCARBON SIGNALING PROTEINS FROM *XENOPUS LAEVIS*.

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Embryos of the African clawed frog, *Xenopus laevis*, are used in FETAX (frog embryo teratogenesis assay-Xenopus) to assess developmental toxicity of chemicals. Halogenated aromatic hydrocarbons, including 2, 3, 7, 8 tetrachlorodibenzo-*p*-dioxin (TCDD), can be potent developmental toxicants. However, several frog species are relatively resistant to TCDD toxicity. Although assessments of TCDD toxicity in X. laevis vary, some reports suggest that this species may also be relatively insensitive. In other vertebrates, TCDD toxicity is mediated by the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor, and by ARNT (aryl hydrocarbon receptor nuclear translocator), the dimeric partner of AHR. To investigate the mechanisms of TCDD sensitivity and resistance in X. laevis, we have isolated cDNA sequences encoding AHR and ARNT from 96 hour embryos. X. laevis resembles many fish in the expression of two distinct AHR genes. However, phylogenetic analysis demonstrates that both X. laevis AHRs are orthologs of mammalian AHR1; neither resembles the AHR2 genes common to fish. The two proteins, AHR1α (836 aa; 94.1 kDa) and AHR1β (833 aa; 93.4 kDa), share 86% amino acid identity, suggesting that they evolved rather recently, perhaps coincident with the tetraploidization of the Xenopus genome approximately 30 mya. X. laevis represents the first species in which multiple AHR1 genes have been observed. Analysis of the single X. laevis ARNT sequence detected revealed that it is an ARNT2 ortholog. ARNT2 is widely expressed in certain fish, but is restricted to kidney and CNS in mammals. Since sequencing multiple X. laevis ARNT clones detected only ARNT2, we suggest that it is the predominant ortholog expressed at this life stage. FETAX is used as a model for predicting the developmental toxicity of materials to other vertebrates. An understanding of the molecular mechanisms underlying sensitivity differences to halogenated aromatic hydrocarbons should contribute to the development of FETAX as a model for developmental toxicity of samples containing these compounds. [NIH: ES11130]

1254 COMPARATIVE STUDY OF HUMAN AND MOUSE AH RECEPTOR FUNCTION USING CHIMERIC RECEPTORS.

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The aryl hydrocarbon receptor (AhR) is a ligand induced transcription factor that exists in the cytoplasm complexed with hsp90 and XAP2. The most widely studied AhR ligand is TCDD. The intra- and inter-species differences in sensitivity to TCDD may occur as a result of differences in receptor structure. Sequence variations in the mouse and human receptors resulting in quantitatively different responses to TCDD are of importance since rodent models are used in *in vivo* toxicity studies. Two chimeras of human and mouse AhR were generated in order to study functional differences due to the low homology in the C-terminal half of the AhR. A unique BamH1 restriction site in both receptors at amino acid residue 424 was used to remove and exchange the C-terminal halves of the receptors. Constructs expressing both FLAG- and yellow fluorescent protein-tagged chimeras were generated. Cellular localization studies in COS-1 cells showed that mouse

AhR (mAhR) is predominantly nuclear, whereas human AhR (hAhR) is cytoplasmic. The mouse-human chimera (MH, murine N-terminus) is cytoplasmic and the human-mouse chimera (HM, human N-terminus) is predominantly nuclear. Co-expression of XAP2 does not affect the localization of hAhR and MH, but causes re-localization of mAhR to the cytoplasm. Interestingly, HM is also unaffected by co-expression of XAP2. All receptors showed ligand induced translocation to the nucleus. Immunoprecipitation of the receptors and western blot analysis demonstrated that each receptor was capable of binding co-expressed XAP2 and endogenous hsp90. Experiments showed that all receptors could induce transcription of a dioxin responsive element (DRE) driven reporter construct and they appear to have similar activity at a saturating ligand concentration. These studies suggest that the C-terminal half of the AhR may influence biochemical properties of the N-terminal half. This study is funded by NIEHS 04869.

1255 DIOXIN ALTERS PROLIFERATION, DIFFERENTIATION AND SENESCENCE IN KERATINOCYTES.

S. Ray. *Dept. of Molecular and Biomedical Pharmacology, University of Kentucky Medical Center, Lexington, KY.* Sponsor: H. Swanson.

TCDD (2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin), a prototype of the polyaromatic hydrocarbons, elicits a variety of toxic, teratogenic and carcinogenic responses in both animals and humans. Many effects of TCDD are mediated by the aryl hydrocarbon receptor (AHR), a bHLH/PAS transcription factor. Ligand activation of AHR alters both cellular proliferation and differentiation of keratinocytes, but the signaling pathways involved in these processes are poorly understood. The objective of this study was to delineate the mechanisms involved in dioxin's tumor promoting activities using normal human keratinocytes (NHK). Using western blot analysis of involucrin and filaggrin expression, markers that are expressed at different stages of differentiation, we have shown that TCDD decreases differentiation of NHKs. This result is supported by flow cytometric analysis, which show that TCDD decreases the number of differentiating keratinocytes while increasing the number of non-differentiating keratinocytes. On performing cell cycle analysis using propidium iodide, we found that treatment with TCDD increases in the number of cells in S-phase and decreases the cells in G1/G0 phase of the cell cycle. By riboprobe protection assay we found that the decrease in differentiation is accompanied by a significant decrease in the expression of $p16^{INK4a}$, p53, Rb, $p27^{KIP1}$ and p14/p15. Some of these results were also verified by western blot analysis. $p16^{INK4a}$ is a cyclin dependent kinase inhibitor that regulates the G1 to S progression and increases both differentiation and senescence. To determine the effect of TCDD on senescence we performed senescence-associated β-galactosidase staining and found that TCDD causes a decrease in senescence of keratinocytes. These results suggest that TCDD may act as a tumor promoter by inhibiting both differentiation and senescence *via* a decrease in the expression levels of $p16^{INK4a}$. Overall these results suggest that the tumor-promoting activities of TCDD is in part mediated by down regulation of these important cell cycle regulatory proteins.

1256 GENERATION OF TEMPERATURE-SENSITIVE LIVER CELL LINES FROM PPARα-DEFICIENT AND WILD-TYPE CB6 MICE.

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Several novel cell lines were generated from the neonatal liver cells of either wild-type (wt) or PPARα-deficient (null) CB6 mice using a temperature sensitive SV40 virus. These cells are passaged at the virus's permissive temperature (34°C) and gene expression experiments are performed at the non-permissive (37°C) temperature. The wt and null cell lines express equivalent concentrations of albumin as well as acyl-CoA oxidase (ACO) and fatty acid binding protein (FABP) mRNA, indicating the retention of liver-specific genes. PPARα presence or absence in the wild type or PPARα-null lines was confirmed by genotype PCR as well as western blotting. The cell lines also retain the ability to appropriately respond to peroxisome proliferators whereby Wy-14, 643 (50mM) treatment for 6 hours failed to induce ACO in the null cells, but significantly increased expression in wt cells. Cell cycle analysis also confirms a defect in the response of PPARα-deficient cells to Wy-14, 643 treatment. These lines will help us to investigate the mechanistic aspects of PPARα-dependent signaling in response to peroxisome-proliferator treatment and effectively recapitulates what has been observed *in vivo* in the mouse models. (Funded by NIH ES07799 and DK49009).

1257 THE EFFECTS OF GREEN TEA EXTRACTS AND INDIVIDUAL TEA CATECHINS ON AhR FUNCTION.

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Previous investigations have implicated green tea as affording protection against polycyclic aromatic hydrocarbon induced cancers in animal models of chemical carcinogenesis. In an effort to understand the mechanism and compound/s responsible for this protection, the effects of green tea extracts (GTE) and individual catechins on the function of the aryl hydrocarbon receptor (AhR) were determined. Green tea (GT) was organically extracted and subsequently fractionated by TLC. The chemical composition of these fractions, as well as a commercially available GTE, were determined by NMR. All GT fractions inhibited tetrachlorodibenzo-*p*-dioxin (TCDD)-induced transcription of a dioxin-responsive element (DRE)-dependent luciferase reporter in stably transfected mouse hepatoma cells in a concentration dependent manner. The most potent of the fractions was then examined for its ability to compete with TCDD for binding to the AhR, and to inhibit TCDD-elicited binding of the AhR to DRE's *in vitro*. From these studies, it was shown that GTE blocked TCDD-induced binding of the AhR to DNA. However, it did not compete directly with TCDD for binding to the receptor. To determine the green tea component/s responsible for the observed effects, individual catechins were tested in these systems at concentrations comparable to those within the GT fractions. Of the catechins tested, epigallocatechingallate (EGCG) followed by epigallocatechin were the most potent antagonists of TCDD induced luciferase, having IC_{50} values of 60uM and 100uM respectively. Subsequent analysis of EGCG's mechanism of action revealed that as seen with GTE, EGCG was capable of inhibiting TCDD induced DRE binding but did not compete with TCDD for binding to the AhR. This data suggests that individual catechins are capable of altering AhR transcriptional activity and that these compounds may function through a mechanism unlike typical AhR antagonists.(Supported in part by NIH grant ES09702, Training Grant ES07026, and Center Grant ES01247)

1258 STRIKING VARIABILITY IN THE STRUCTURES OF ARNT AND ARNT2 IN THE RAT.

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The biological and toxic effects of dioxins are largely mediated by the aryl hydrocarbon receptor (AHR). The AHR functions as a ligand-activated transcription factor binding DNA as a heterodimeric complex with a structurally related bHLH/PAS protein, the AHR nuclear translocator (ARNT) or its homologue in the brain, ARNT2. We recently discovered 3 splice variants of AHR in an extremely TCDD-resistant rat strain, Han/Wistar (Kuopio), which appear to account for its resistance. In ARNT protein, so far only one splice variant (deletion of exon 5) has been described in the literature. To find out whether structural deviations of ARNT could be related to TCDD sensitivity, we cloned hepatic ARNT and hypothalamic ARNT2 cDNAs by blunt-end cloning after RT-PCR from untreated H/W and TCDD-sensitive Long-Evans (Turku AB) rats. To our surprise, we recorded several different products of alternative splicing of ARNT mRNA: in addition to exon 5, deletions occurred at 3' end of exon 6 (NTs 354-494) and at 5' end of exon 11 (NTs 964-978). A small insertion (CAG) was detected at the 5'end of exon 20. Interindividual variation transpired in the number of GCA repeats at the 5' end of exon 16. In the case of ARNT2, an insertion of 31 NTs appeared at NT 2128. Importantly, this insertion contained a premature stop codon resulting in a truncated protein at the C-terminus, with potentially a repressor-type function. All other variants represented in-frame changes, but at least the two largest deletions are likely to seriously impair protein function. There were no major differences between the two rat strains in relative expression levels of the variants as assessed by semi-quantitative RT-PCR implying that they do not contribute to strain-specific sensitivity to TCDD.

1259 THE ARYL HYDROCARBON RECEPTOR (AHR) HOMOLOGUE OF THE SOFTSHELL CLAM (*MYA ARENARIA*) DOES NOT BIND PROTOTYPICAL AHR LIGANDS TCDD OR BNF.

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The aryl hydrocarbon receptor (AHR) mediates various toxic effects of aromatic hydrocarbon compounds, including 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD). Some populations of softshell clams (*Mya arenaria*) have historically exhibited a high prevalence of gonadal neoplasia. Although the etiology of these germinomas is unknown, their incidence has been correlated with environmental herbicide (TCDD-contaminated) usage. One hypothesis proposes an AHR-mediated pathway of tumorigenesis. To investigate this, a clam AHR homologue (cDNA) was cloned. Amino acid alignments indicate domains characteristic of vertebrate AHRs. Despite these structural similarities, binding analyses using *in vitro*-expressed clam AHR or clam cytosolic proteins showed that this AHR homologue

binds neither [^{3}H]TCDD nor [^{3}H]B-naphthoflavone (BNF). However, preliminary studies indicate the *in vitro*-expressed clam AHR does exhibit sequence-specific interactions with a mammalian xenobiotic response element (XRE) in the absence of exogenous ligand. *in vitro*-expressed *D. melanogaster* and *C. elegans* AHR homologues also lack specific binding to [^{3}H]TCDD and [^{3}H]BNF. The lack of specific binding to these prototypical AHR ligands appears to be a property shared by known invertebrate AHR homologues, distinguishing them from vertebrate AHRs. Comparative studies of phylogenetically diverse organisms may help identify an endogenous ligand(s) and the physiological role(s) for this protein.

1260 HORMONAL ACTIVATION OF LACTATE DEHYDROGENASE GENE EXPRESSION IN BREAST CANCER CELLS.

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In previous studies, we identified the -92 to -37 region of the lactate dehydrogenase (LDH) gene promoter as estrogen (E2)-responsive in transient transfection studies in MCF-7 human breast cancer cells. This promoter sequence contains a cAMP response element (CRE) and nuclear extracts from MCF-7 cells bound [^{32}P]-58/-36 (CRE-sequence from LDH promoter) in gel mobility shift assays to form a broad retarded band which was decreased in intensity after competition with an unlabeled consensus CRE oligonucleotide. The retarded band was supershifted after incubation of nuclear extracts with antibodies that bound ATF-1 and CREB. Constitutively active protein kinase A and an inducer of cAMP (cholera toxin) induced reporter gene activity in breast cancer cells transfected with the E2-responsive pLDH1 construct (contains the -58 to +9 LDH gene promoter insert) confirming that cAMP-PKA induction also activates the CRE. Hormonal activation of pLDH1 and related constructs was also inhibited by cotransfection with KREB, a dominant negative form of CREB. Activation of CREB and ATF-1 by E2 through non-genomic pathways was confirmed in studies showing that 10 nM E2 caused a 7- and 27-fold induction of reporter gene activity MCF-7 cells transfected with GAL4-ATF1 and GAL4-CREB fusion proteins and a construct containing five tandem GAL4 response elements. Thus, LDH upregulation by E2 is due to non-genomic activation of the cAMP-PKA pathway. (Supported by NIH ES09253 and ES09106)

1261 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) INDUCES PLASMINOGEN ACTIVATOR INHIBITOR-1 THROUGH AN ARYL HYDROCARBON RECEPTOR-MEDIATED PATHWAY IN A MOUSE HEPATOMA CELL LINE.

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2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), an ubiquitous environmental pollutant, elicits a variety of toxicities and is a well-known carcinogen. TCDD alters the expression of many genes including CYP1A1/2, CYP1B1, glutathione S-transferase Ya, aldehyde-3-dehydrogenase, NAD(P)H:quinone oxidoreductase, TGF-alpha, TGF-beta, plasminogen activator inhibitor-2 and interleukin-1beta. The present study investigated the effect of TCDD on plasminogen activator inhibitor-1 (PAI-1) in a mouse hepatoma cell line (Hepa1c1c7). Based on Western and Northern blots, TCDD induced dose- (0, 0.1, 1 & 10 nM) and time- (0, 3, 6, 12, 24 & 48 h) dependently PAI-1 in Hepa1c1c7 cells. However, TCDD did not induce PAI-1 in the aryl hydrocarbon receptor (AhR) and AhR nuclear translocator (Arnt)-deficient mutants derived from Hepa1c1c7 cells, indicating a functional role of the AhR-Arnt complex in this effect. Transfection with PAI-1 promoter resulted in increased PAI-1 promoter activity in Hepa1c1c7 cells treated with TCDD, but no such effect occurred in the AhR or Arnt-deficient cell lines, implying involvement of the AhR and Arnt proteins. In addition, alpha-naphthoflavone and phenanthroline, two AhR antagonists, blocked the enhancing effect of TCDD on PAI-1 promoter-coupled luciferase activity in Hepa1c1c7 cells. The results revealed that the induction of PAI-1 by TCDD occurs in the same dose range as other known TCDD-induced AhR-mediated signal transductions, implicating PAI-1 as the most recently found gene induced by TCDD.

1262 HYPOXIA INDUCES PROTEASOME-DEPENDENT DEGRADATION OF ESTROGEN RECEPTOR αA (ERα) IN HUMAN BREAST CANCER CELLS.

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Cell growth under hypoxic conditions induces a cascade of responses linked to changes in enzymes responsible for metabolic adaptation for growth and survival under low oxygen conditions. Hypoxic conditions in tumors can lead to a more clinically aggressive phenotype and, therefore, we have investigated the effects of reduced oxygen levels on key regulatory genes/proteins in estrogen receptor α (ERα)-positive ZR-75 human breast cancer cells. ZR-75 cells were maintained in an atmosphere of 21% (normoxia) or 1% (hypoxia) oxygen or grown in the presence of 0-500 μM cobaltous chloride to approximate hypoxic conditions. Western blot analysis of whole cell lysates from ZR-75 cells grown under normoxia or hypoxia for up to 24 hr showed that levels of several nuclear transcription factors, including Sp1, Sp3, hypoxia inducible factor 1β (HIF1β or Arnt) and the aryl hydrocarbon receptor (AhR), were relatively unchanged. In contrast, HIF1α protein was not detected under normotic conditions, but accumulated to very high levels between 3 - 24 hr after exposure of cells to 500 μM cobaltous chloride or 1% O_2 treatment. This increase in HIF1α was due to inhibition of proteosome-dependent degradation of HIF1α under normoxic conditions. In contrast, growth of ZR-75 cells in 500 μM cobaltous chloride or 1% oxygen resulted in > 70% degradation of ERα protein and, in time course studies, this response was observed after growth for > 3 hr in hypoxic conditions. Treatment with the proteasome inhibitor MG132 did not affect levels of ERα protein in cells maintained in 21% oxygen, whereas hypoxia-induced degradation of ERα protein was reversed by the proteasome inhibitor. The role of estrogens and antiestrogens on ERα protein and hormone-induced transactivation is complex and dependent, in part, on "optimal" expression of ERα protein. (Supported by NIH ES04176 and ES09106)

1263 NOVEL MECHANISM OF TCDD TOXICITY: SEQUESTRATION OF ARNT FROM AN ENDOGENOUS ROLE IN P53 GENE INDUCTION.

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Most of the toxic and carcinogenic actions of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), a ubiquitous environmental contaminant, are mediated by its ability to bind and activate the aryl hydrocarbon receptor (AhR). The physiological effects of TCDD exposure include altered drug metabolism, disruption of endocrine signaling, and tissue-specific alteration of cellular processes including proliferation, differentiation, and apoptosis. Indeed, AhR directly mediates the gene induction of several xenobiotic-metabolizing enzymes *via* ineracting with its DNA binding partner, ARNT; however, the mechanisms by which AhR mediates many of the TCDD effects is poorly understood. One mechanism by which the AhR may mediate TCDD toxicity includes the sequestering of ARNT from an endogenous role. ARNT is a promiscuous nuclear transcription factor that not only homodimerizes but also dimerizes to transcription factors of the hypoxia signaling pathway (HIF-1a) and neurogenesis (Sim). To investigate the putative role of ARNT in modulating other signaling pathways, we performed transient transfection assays using a reporter gene regulated by the P53 promoter and c-myc and ARNT expression plasmids. In addition to our discovery that ARNT is able to enhance c-myc induction of the P53 gene, we have also discovered that this occurs independent of DNA binding by ARNT. In another approach, we have analyzed endogenous P53 mRNA levels within stably transformed MCF7 cells that over-express ARNT, c-myc or both. Our data indicates that TCDD toxicity may be mediated by activation of AhR, which sequester ARNT, impairs the induction of P53, and accelerates cell cycle progression and/or inhibits apoptosis.

1264 INTERACTION OF AH RECEPTOR WITH PHENOLIC ANTIOXIDANT SIGNAL TRANSDUCTION.

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The aryl hydrocarbon receptor (AhR) mediates a spectrum of adaptive and toxic responses to the environmental contaminant TCDD and related halogenated aromatic hydrocarbons. AhR may also play a role(s) in development, growth, and differentiation of tissues in the absence of an exogenous ligand. The broad range of TCDD toxicity and AhR function suggest that the mechanism of AhR action involves multiple signaling mechanisms. In this study, we examined the interaction of AhR with phenolic antioxidant signal transduction, which involves oxidative signaling. Phenolic antioxidants, such as tert-butylhydroquinone (tBHQ), hydroquinone (HQ), or catechol, induce the expression of CYP1A1 in mouse hepa1c1c7 cells; the induction is enhanced by inhibition of protein synthesis by cycloheximide (CHX) (termed superinduction). Induction by the antioxidants is both concentration and time-dependent. Furthermore, the induction and superinduction require AhR and Arnt, as they are absent in AhR or Arnt defective variant cells, and are mediated through the DRE-containing enhancer of CYP1A1, as they are reconstituted in a CYP1A1 enhancer-luciferase reporter expression system. These findings demonstrate that phenolic antioxidants can activate AhR-mediated gene transcription and suggest interaction of AhR with antioxidant mediated oxidative signaling.

1265 TRANS-ACTIVATION OF PPARα AND PPARγ BY PHTHALATE MONOESTERS.

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Phthalate esters are widely used as plasticizers in the manufacture of products made of polyvinyl chloride and can be found in a variety of industrial products, including fixatives, detergents, and solvents. Rodent hepatocarcinogenesis, in part linked to the activation of peroxisome proliferator activated receptor-α (PPAR), has been reported for mono-2-ethylhexyl phthalate (MEHP). Recent studies demonstrate substantial human urinary levels of several other phthalate monoesters, including monobenzyl phthalate (MBzP) and monobutyl phthalate (MBuP), raising the question of whether significant PPAR activation, and perhaps adverse health effects, accompanies environmental or occupational exposure to these agents. The responsiveness of mouse and human PPARα and PPARγ to activation by phthalate monoesters (0-300 μM) was therefore investigated by transient transfection using COS-1 cells. Treatment with MBzP or MBuP for 24 hr increased mouse PPARα transcriptional activity by 3 to 3.5-fold. The activation of human PPAR was somewhat weaker, suggesting a species-dependence of PPARα activation by these chemicals. Mouse PPARγ transcriptional activity was increased 2-3-fold by MBzP and MBuP, while the activation of human PPARγ was again weaker. The activation of PPARα by MBzP and MBuP was confirmed using PPARα-stably transfected FAO liver cells, where the endogenous PPARα target genes acyl-CoA oxidase and 3-ketoacyl-CoA thiolase were induced by both phthalate monoesters in a PPARα-dependent manner. We conclude that MBzP and MBuP are environmental chemical activators of PPARα and PPARγ, and perhaps may contribute to phthalate toxicity. Further study is required to characterize the effects of these phthalate monoesters on PPAR target genes *in vivo* and to evaluate their potential for adverse human health effects. [Supported in part by NIH/NIEHS grants F32 ES11105 (CHH), ES07381 (DJW), and Superfund Basic Research Center at Boston University].

1266 TIMING OF TCDD TREATMENT WITH RESPECT TO HORMONAL STIMULATION DICTATES MAPK SENSITIVITY OF THE AHR BLOCK ON ADIPOGENESIS.

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The mouse embryo fibroblast cell line C3H10T1/2 is a multipotential cell population that will differentiate into various cell types under the appropriate conditions. 10T1/2 cells will undergo adipogenesis if treated with insulin, dexamethsone, methylisobutylxanthine, and the PPAR*gamma* ligand BRL49653 (IDM/BRL). The extent of adipogenesis is measured by the expression of the key adipogenic transcription factor PPAR*gamma* and the number of mature adipocytes at day 8. We use the *gamma*1-isoform of PPAR (PPAR*gamma*1)expression as a marker of the commitment phase of adipogenesis. We have previously shown that if cells are challenged with TCDD 48hrs prior to adipogenic stimulation, there is a block on adipogenesis that is completely dependent on extracellular signal-regulated kinase (ERK) activation for 6-16hrs after IDM/BRL stimulation. The use of either of the MEK inhibitors PD98059 or UO126 reversed the TCDD-mediated inhibition when added 6-16hrs after IDM/BRL, thus demonstrating there is an ERK sensitive component of the TCDD mediated inhibition. Here we show that adding TCDD substantially after IDM/BRL treatment gives increased PPAR*gamma*1 suppression and loss of adipogenesis. The addition of TCD 6-12hrs after IDM/BRL yields an 80% reduction of PPAR*gamma*1 expression, which again is fully reversed by subsequent addition of MEK inhibitors. Here we define the timing of MEK activation that is necessary for this late suppresion of PPAR*gamma*1 expression by TCDD *via* AhR and the mechanism of cross-talk between the two pathways.

1267 REGULATION OF THE HUMAN *UGT1A1* GENE BY THE NUCLEAR PREGNANE X RECEPTOR.

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The UDP-glucuronosyltransferase, *UGT1A1*, controls the detoxification of bilirubin and plays a role in geno-protection against carcinogens such as N-hydroxy 2-amino-1-methyl-6-phenylimidazo[4, 5-b]pyridine. Nuclear orphan receptors have recently been shown to regulate metabolic pathways in response to chemical and hormone exposure by alteration in gene statement patterns of drug-metabolic enzymes. Here, we demonstrate that *UGT1A1* was inducible by a number of compounds known to be Pregnane X receptor (PXR) ligands. HepG2 cells that over-express the human PXR confirm that rifampicin and dexamethasone induce *UGT1A1* gene statement as demonstrated by enhanced mRNA levels as well as catalytic activities. To examine the molecular interactions that control PXR-medicated *UGT1A1* statement, the *UGT1A1* responsive elements were identified and cloned into reporter plasmids. A 230-bp responsive fragment, localized approximately ¡V3.5 kb upstream of the transcriptional start site, was required for PXR-mediated induction as examined in transfection experiments. In addition, a constitutively active PXR statement plasmid called VP16-hPXR stimulated enhancer-dependent reporter gene activity, producing a 10-fold induction of luciferase activity, thus confirming that the PXR-distal *UGT1A1* responsive sequence interaction is functional. Taken together, these observations demonstrate a regulatory mechanism for *UGT1A1* induction that is dependent upon nuclear receptor PXR. The induction of *UGT1A1* through PXR may provide a defensive mechanism against the toxicity of endogenous and foreign chemicals. (Supported in part by USPHS grant GM49135).

1268 LIGAND-INDEPENDENT PHYSIOLOGICAL MECHANISMS FOR AHR ACTIVATION.

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The aryl hydrocarbon receptor is a ligand-activated basic helix-loop-helix (bHLH)/Per-Arnt-Sim domain transcription factor. Although the mechanism of AhR activation by xenobiotics is well understood, activation of AhR in the absence of exoligand remains unclear. Here we have demonstrated an alternate regulatory mechanism of AhR activation in the absence of exogenous ligand in both suspended and monolayer cells using mouse embryonic fibroblast (MEF) cell lines which contain integrated mouse Cyp1B1 promoter constructs linked to luciferase reporters responding to AhR activation. First, the AhR activity was increased in methylcellulose-suspended fibroblasts as effectively as by TCDD in confluent monolayer. Second, overlay of monolayer fibroblasts with methylcellulose substantially increased AhR activity while retaining adhesion. Suspension stimulated AhR activity was completely insensitive to an antagonist, a-naphthoflavone (aNF) indicating that activation is not due to a constitutive ligand. Suspension activation was selectively inhibited by Ro 32-8220, a PKC inhibitor and activated by LiCl, a GSK-3b inhibitor. We project that these inhibitors target the suspenson activation of AhR. Several specific cellular inhibitors affect each process similarly (Thapsigargin, MG132, and geldanamycin). These data characterize a ligand-independent physiological process for AhR activation.

1269 GENETIC EVIDENCE THAT THE P23 CO-CHAPERONE REGULATES HUMAN DIOXIN RECEPTOR SIGNALING IN A YEAST MODEL SYSTEM.

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The dioxin (or aryl hydrocarbon) receptor is a nuclear receptor that binds toxic ligands (e.g., TCDD) and it requires the action of Hsp90 and cofactors for proper function. The dioxin receptor exists in a cytoplasmic complex which contains a dimer of Hsp90, other co-chaperone proteins, and p23 prior to ligand exposure. Whether p23 has a role in dioxin receptor signaling is not known. *in vitro* studies suggest that the p23 co-chaperone protein enhances the stability of dioxin receptor-Hsp90 complexes following ligand binding. This stabilization may involve p23's ATP-dependent interaction with the nucleotide-binding site of Hsp90. We used a yeast genetic model system that expressed functional human dioxin receptor and Arnt proteins to test whether p23 affects dioxin receptor signaling. Deletion of the SBA1 gene (the yeast p23 homolog) reduced ligand-mediated dioxin receptor signaling in a reporter gene assay and reduced ligand potency by approximately five-fold. Signaling and ligand potency were restored to wild type levels when SBA1 was expressed in this strain. More importantly, human p23 effectively substituted for yeast p23 in this assay. To further investigate the role of p23 in dioxin receptor signaling, we used a temperature sensitive Hsp90 mutant strain with a severe dioxin receptor signaling defect both at the permissive and non-permissive temperatures. The mutation in this hsp90 strain maps to the ATP/p23 binding pocket, suggesting that the dioxin receptor signaling defect and temperature sensitivity may be due to impaired p23-Hsp90 interactions. In support of this hypothesis, we found that SBA1 or human p23 overexpression suppressed temperature sensitivity and the dioxin receptor signaling defect in this mutant. These results provide the first genetic evidence that p23 modulates dioxin receptor signaling and suggest that p23 levels may be a determinant of ligand-mediated toxicity by TCDD and related compounds.

1270 FUNCTIONAL ANALYSIS OF AH RECEPTORS CONTAINING MUTATIONS WITHIN THE NUCLEAR LOCALIZATION SIGNAL (NLS).

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The nuclear localization signal (NLS) of the Ah receptor (AHR) spans amino acids 12-39 and overlaps the basic region that is involved in DNA binding. Studies were initiated to evaluate the importance of the NLS in AHR localization, AHR degradation, and AHR-mediated gene induction. Stable cell lines were generated in the

Hepa-1 cell line that expressed 1) wild type AHR (wtAHR), 2) AHR containing a K to A substitution at amino acid 13 (NLS-1) and 3) AHR containing an R to A substitution at amino acid 12 and a K to A substitution at amino acid 13 (NLS-2). Expression of NLS-1 or NLS-2 *in vitro* revealed that both proteins could bind to DNA in a TCDD-dependant manner to the same level as wtAHR. Immunohistochemical analysis of the NLS-1 and NLS-2 stable cell lines showed that the mutated AHRs were predominantly cytoplasmic and did not translocate to the nucleus in the presence of TCDD. However, the staining intensity of the NLS-1 and NLS-2 became reduced following exposure to TCDD for 4-16 hours. These results were confirmed by Western blot analysis of various AHR mutants and showed that NLS-1 was reduced by 60-80% following 16hr TCDD exposure while NLS-2 was reduced by approximately 50% after 16hr TCDD exposure. Interestingly, while neither NLS-1 nor NLS-2 could be detected in the nucleus, cells expressing these proteins exhibited TCDD-mediated *CYP1A1* induction that was 10-20% of that observed in cells expressing wtAHR or in the wild type Hepa-1 line. Thus, these results support the hypothesis that AHR can be proteolytically degraded within the cytoplasmic compartment, but also suggest that the NLS-1 and NLS-2 mutations are not absolute in blocking nuclear localization. Supported by ES10991.

1271 ANALYSIS OF RAINBOW TROUT AH RECEPTOR ISOFORMS (RTAHR2) IN CELL CULTURE REVEALS CONSERVATION OF FUNCTION.

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Two distinct aryl hydrocarbon receptor (AHR) cDNAs have been isolated from rainbow trout. These proteins termed rtAHR2 α and rtAHR2β are 97% identical at the amino acid level but are reported to have distinct functions with regard to AHR-mediated gene regulation when expressed in COS cells. To evaluate this hypothesis, these proteins were functionally evaluated *in vitro* and in the Chinese hamster lung cell line, E36. To facilitate the ability to detect and measure the expression of these proteins, both rtAHR2 isoforms were tagged with the FLAG peptide on the carboxy-terminus. When the rtAHR2 proteins were expressed *in vitro* and then incubated with 2, 3, 7, 8-tetrachlorodibezo-*p*-diozin (TCDD) in the presence of either mammalian or rainbow trout ARNT, they failed to form functional complexes that could associate with xenobiotic response elements (XRE) in a ligand-dependant manner. Use of different temperatures, protein concentrations or extracts from trout cells did not affect the negative results. In contrast, both proteins exhibited positive function on AHR-mediated signaling when expressed in E36 cells. Both rtAHR2 isoforms showed a cytoplasmic distribution in the unliganded state and could drive expression of a reporter gene under control of the trout *CYP1A3* promoter. Although both proteins could induce reporter gene activity to the same magnitude, the EC_{50} values of the two isoforms for TCDD differed by an order of magnitude with the rtAHR2β isoform less responsive to TCDD. When function of the rtAHR2 isoforms were tested in the context of the dominant negative $rtARNT_a$ isoform, TCDD-mediated induction of reporter gene activity was significantly reduced compared to when $rtARNT_b$ was utilized. Thus, both rtAHR2 isoforms appear to exhibit positive function in AHR-mediated signaling suggesting conservation. Supported by ES 08890.

1272 A SINGLE AMINO ACID DIFFERENCE UNDERLIES THE DIFFERENTIAL TRANSACTIVATION ACTIVITIES OF THE RAINBOW TROUT ARYL HYDROCARBON RECEPTORS (RTAHR2α AND RTAHR2β).

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Two rainbow trout aryl hydrocarbon receptor cDNAs (rtAhR2α and rtAhR2β) were recently isolated and characterized. The predicted amino sequences share 98% amino acid identity yet their functional properties differ. Both rtAhR2α and rtAhR2β bind 2, 3, 7, 8 tetrachlorodibenzo-*p*-dioxin (TCDD), dimerize with rainbow trout ARNTb (rtARNTb), and recognize dioxin response elements *in vitro*. However, in a transient transfection assay in COS-7 cells the proteins show differential activity towards a reporter driven by the rainbow trout cyp1a promoter. In response to 10 nM TCDD, cells co-transfected with rtARNTb and rtAhR2α showed an 8.6-fold increase in luciferase activity whereas cells co-transfected with rtARNTb and rtAhR2β showed only a 1.5-fold increase. Our objective was to determine which of the amino acid substitutions are responsible for the differences in rtAhR2α and rtAhR2β transactivation activity. Chimeric rtAhRs were constructed by switching sequences between the two rtAhR2s and an N-terminal region was responsible for the differential transactivation activity of the proteins. To determine the specific residues underlying the differential activity we utilized site-directed mutagenesis to convert certain rtAhR2β amino acids to those found in rtAhR2α. When amino acid 111 in rtAhR2β, located just N-terminal to the PAS domain, was changed from lysine to glutamate, transactivation activity of the altered rtAhR2β was similar to that of rtAhR2α. When substitutions are made in the zebrafish and human AhRs at this residue, activity is similarly altered indicating that this amino acid is essential for the structure and function of the aryl hydrocarbon receptors.

1273 DO DIFFERENCES IN THE DEER MOUSE AH RECEPTOR LIGAND BINDING DOMAIN EXPLAIN WHY DDE IS A CYP1A1 INDUCER IN THIS SPECIES?

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Previously, we reported that DDE exposure in the deer mouse induces ethoxyresorufin-deethylase activity and that this activity is neutralized by rat anti-CYP1A1 antibody but not rat anti-CYP2B antibody. This was accompanied by an increase in CYP1A1 message as determined by RT-PCR. In contrast, exposure of laboratory rat and mouse to DDT or DDE results in CYP2B induction, a phenomena not observed in the deer mouse, a New World species. Induction of CYP1A1 by environmental chemicals predominantly involves binding of the compound to the Ah receptor, resulting in its activation, dimerization with ARNT, and binding of the complex to dioxin response elements in the 5' flanking region of the CYP1A1 gene. DDE has been shown to bind with the murine Ah receptor. One hypothesis for the ability of DDE to induce CYP1A1 in the deer mouse is that the deer mouse Ah receptor differs significantly in the ligand binding domain from laboratory mouse. To test this hypothesis, we isolated mRNA from deer mouse liver, produced Ah receptor and ARNT cDNA by RT-PCR, and sequenced the resulting clones. The most significant difference in the Ah receptor was in position 236 in which the deer mouse has a valine and the house mouse a methionine. Other differences were in position 306 (valine to isoleucine), 317 (lysine to argenine), 329 (methionine to isoleucine) and 331 (tyrosine to histidine). The deer mouse Ah receptor binding domain also differed from the rat in positions 236, 306, 316, and 331. We are currently in the process of determining the effect of each of these substitutions on xenobiotic binding.

1274 MOUSE HEPATOCYTE RESPONSE TO PEROXISOME PROLIFERATORS: DEPENDENCY ON HEPATIC NONPARENCHYMAL CELLS AND PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR α (PPARα).

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Peroxisome proliferators (PPs) are rodent nongenotoxic hepatocarcinogens that induce peroxisome proliferation and DNA synthesis, and suppress apoptosis in rodent hepatocytes. PPs act through the PP activated receptor α (PPARα). Tumour necrosis factor α (TNFα) and hepatic nonparenchymal cells (NPCs), the major source of TNF α in the liver, have also been implicated in mediating the rodent hepatic response to PPs. We have used an established *in vitro* model to investigate the interaction between PPARα and NPCs in regulating the response to PPs. Using normal hepatocyte cultures containing around 20% NPCs, the PP nafenopin (50μM) induced DNA synthesis and suppressed TGFβ1 -induced apoptosis. However, when the NPCs were removed by differential centrifugation, nafenopin did not induce DNA synthesis nor suppress apoptosis in the pure hepatocytes. Reconstitution of the normal hepatocyte cultures by mixing together the pure hepatocytes and the previously separated NPCs in the same proportions as the original cell preparation (17.7 + 8.7% NPCs) restored these responses to nafenopin. Interestingly, nafenopin was still able to induce β-oxidation in the pure hepatocyte cultures, consistent with NPCs being required for PP-induced growth but not for peroxisome proliferation. Next, we evaluated the role of PPARα in the hepatocyte dependency upon NPCs. Interestingly, NPCs isolated from PPARα null mice, like those isolated from the wild type NPCs, restored the hepatocyte response to nafenopin. However, as expected, PPARα null hepatocytes remained non-responsive to PPs, irrespective of the genotype of the added NPCs. These data support a role for NPCs in facilitating a response of hepatocytes to PPs that is ultimately dependent on the presence of PPARα in the hepatocyte.

1275 DOWNREGULATION OF LACTOFERRIN BY PPARα LIGANDS ASSOCIATED WITH THE RODENT HEPATOCARCINOGENICITY OF PEROXISOME PROLIFERATORS.

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In rats and mice, peroxisome proliferators (PPs) cause liver cancer, associated with suppression of apoptosis and increased hepatocyte replication. PPARα (PP activated receptor α) is a ligand-activated transcription factor that mediates the growth

and tumorigenic responses to PPs *via* mechanisms that remain to be identified. Using microarray gene expression profiling we detected a 3 to 7-fold down-regulation of murine hepatic lactoferrin (LF) in response to the PP, diethylhexylphthalate (DEHP; 1150mg/Kg). LF is an iron binding glycoprotein implicated in cancer and in regulating inflammatory responses, possibly *via* tumor necrosis factor α (TNFα). Northern blot analyses using a human LF probe confirmed down-regulation of LF mRNA by DEHP. LF has been reported to repress TNFα levels suggesting that LF down-regulation by PPs may permit TNFα levels to rise, enhancing hepatocyte survival and proliferation. To address this, we asked if LF down-regulation could drive the perturbation of hepatocyte growth by PPs. *in vitro*, MEHP (the principal metabolite of DEHP and proximal PP), the PP nafenopin (50μM) or exogenous TNFα (5000U/ml) induced hepatocyte proliferation and suppressed apoptosis. LF (200μM) blocked the growth but not the peroxisome proliferation response to MEHP and nafenopin but could not block the growth response to TNFα. These data suggested a role for LF in the growth response to PPARα ligands. However, sequence similarities raised the possibility that the DEHP-regulated mRNA could be transferrin, a related gene previously shown to contain a PP response element (PPRE). Immunocytochemistry using specific anti-LF and anti-TF antibodies demonstrated that both LF and TF are expressed in murine liver and furthermore, both were downregulated by DEHP in both wild type and PPARα null mouse liver. These data suggest that the regulation of iron binding proteins by PPARα ligands plays a role in PP-mediated liver growth, but not in peroxisome proliferation.

1276 HEPATIC EFFECTS OF PPARδ ARE DISTINCT FROM PPARα

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The PPAR receptor family includes three receptor subtypes PPARα, PPARγ, and PPARδ, which are all members of the steroid/thyroid/retinoid nuclear receptor superfamily of transcription factors. PPARα is abundant in rodent liver and plays a role in regulation of fatty acid metabolism. PPARδ is moderately expressed in most tissues including liver, but its biological function is unclear. PPARδ agonists have been reported to lower lipids in animal models; however, it is not known if this effect is distinct from activation of PPARα. We used a potent and selective PPARδ agonist (GW501516) to compare the effects of activation of PPARδ to those induced with PPARα agonists (GW7647 and fenofibrate). In *in vitro* assays, high concentrations of GW501516 cross-react with PPARα receptors. Four days of oral administration of low and medium doses of GW501516 to Wistar Han rats did not cause peroxisome proliferation (PP), but PP occurred in rats that received a high dose. We hypothesized that induction of PP induced by GW501516 in rodents was due to activation of PPARα consistent with liver levels of GW501516 at the high dose. Gene expression analyses support this hypothesis in that peroxisomal bifunctional enzyme mRNA levels were increased in only the rats that received the high dose of GW501516 and in rats that received both PPARα agonists. Serum triglycerides decreased in all treated groups with the largest changes occurring in rats that received the high dose of GW501516 and both PPARα agonists. Lipid lowering was seen at doses below those that resulted in the induction of PP. Increase in liver weight was seen only in rats given the high dose of fenofibrate; although, a dose related increase of hypertrophy and granular eosinophilia was seen in the livers from GW501516- and fenofibrate-treated rats. Results suggest that PP caused by GW501516 results from activation of PPARα and that the effects of GW501516 on lipid lowering are independent of the induction of PP.

1277 PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR ALPHA IS ESSENTIAL FOR LIVER REGENERATION FOLLOWING PARTIAL HEPATECTOMY.

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Liver regeneration following partial hepatectomy involves several signaling mechanims including activation of the small GTPases Ras and RhoA 1, 2 in response to mitogens 3, leading to DNA synthesis and cell proliferation. Peroxisome proliferator activated receptor alpha (PPARa) regulates the expression of several key enzymes in isoprenoid synthesis 4, 5, key events for membrane-association of Ras and RhoA. Thus, the role of PPARa in cell proliferation was tested. Peak DNA synthesis 42 hours after partial hepatectomy was reduced by ~ 60% in PPAR*alpha* deficient mice compared to wild-type mice, despite increases in TNF*alpha* and IL-1 in both groups. After partial hepatectomy, PPAR*alpha* regulated genes, farnesyl pyrophosphate synthase and HMG-CoA synthase, were significantly increased in wild-type but not in PPAR*alpha*-/- mice. Also, under these conditions, membrane-association of Ras and RhoA was high in wild-type mice but was nearly absent in PPAR*alpha*-/- mice. Concomitantly, Ras and RhoA were elevated in the cytosol in PPAR*alpha*-/- mice. Deletion of PPAR*alpha* blunted the degradation of cell cycle inhibitors p21waf and p27kip1, prevented activation of cdk2 and cdk4, and blunted phosphorylation of Rb protein. These results support the hypothesis that PPAR*alpha* is necessary for cell cycle progression in regenerating mouse liver *via* mechanisms involving Ras and RhoA.

1278 THE COMMON MARMOSET (CALLITHRIX JACCHUS) IN DRUG SAFETY STUDIES.

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Callitrichids are not adapted to captivity, except common marmosets, for which a database is now available in toxicology, allowing regulatory submission. Animals of defined health status, age and history are available since purpose-bred colonies are established. We have defined background data on clinical pathology, electrocardiology, ophthalmology and histopathology facilitating interpretation of toxicology studies. Marmosets are the smallest non-rodents used in toxicology and have therefore reduced dietary and particularly substance requirements. They can thus be employed early in the drug development process. Their small size was not always an advantage because of limited amounts of blood which can be taken on studies, and because of difficulties in performing some examinations (e.g. ophthalmology). Blood analysis is now more easily possible with microtechniques, except for toxicokinetics. In this case satellite animals are recommended in order to not jeopardize results. The eye is small, deep-seated and eyelids are difficult to maintain open, making fundus photographs difficult, but possible for experienced ophthalmologists. Marmosets are not yet used routinely in reproductive toxicology, although presenting several advantages: rapid maturation, short pregnancy, and multiple births. The main disadvantage consisted of the absence of external signs of the ovarian cycle preventing to detect gestation early enough without repeated blood withdrawals. Timing of ovulation and pregnancy can however be determined by monitoring urinary or fecal hormones. In addition, similarity to simian primates suggests that the marmoset should be a good experimental model, suitable for teratological investigations. Several reproductive biology studies have been performed. Unfortunately, a large database is not yet available for reproductive toxicology studies. If the use of the marmoset could be more often envisaged, for example to possibly replace the cynomolgus monkey in reproductive toxicology, a larger database could be built and, as a consequence, this species would be more easily accepted as an alternative.

1279 A COMPARISON OF SELECTED LABORATORY INVESTIGATION PARAMETERS IN CYNOMOLGUS MONKEYS FROM MAURITIUS AND CHINA.

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The limited availability of primates for pre-clinical safety evaluation studies is a continuing issue. Huntingdon Life Sciences has accumulated background data from animals supplied from Mauritius over many years, but more recently other sources have been investigated. Yunnan in China has been identified as a suitable source and a number of projects have been completed with these animals. The purpose of this review is to compare results for the parameters normally investigated in the laboratory investigations conducted during pre-clinical toxicity tests; haematology, blood chemistry and urinalysis from the two sources. While review of blood chemistry and urinalysis data from animals from the two sources indicated few clear consistent differences in any of the standard parameters included in safety evaluation studies, there were some marked differences seen in the haematology parameters. This was most apparent in erythrocyte values. The potential for differences in haemoglobin parameters in cynomolgus monkeys from different sources is a possible confounding factor in the interpretation of the results of toxicological studies. It is concluded that the source of cynomolgus monkeys must be taken into account in the interpretation of haematological data and in fact, ideally, cynomolgus monkeys from the same source should be used throughout a programme of work on the same compound.

1280 EFFECTS OF A SINGLE INTRAMUSCULAR INJECTION OF KETAMINE IN CYNOMOLGUS MONKEYS.

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Ketamine is frequently used to produce temporary chemical restraint in primates for procedures performed during toxicology studies. The cynomolgus monkey is a common species used on these studies. However, limited information is available on the acute effects of ketamine administration on the various parameters evaluated during toxicology studies. Effects of ketamine administration on parameters typically evaluated on these studies could confound the interpretation of the data.

Thus, the effects of ketamine were evaluated in cynomolgus monkeys. Following IM administration of 10 mg/kg of ketamine or 0.9% saline, monkeys were evaluated for clinical effects including onset and recovery from sedation as well as food intake. Clinical pathology parameters including hematology, coagulation, platelet aggregation, serum chemistry and urinalysis/urine chemistry were also determined at multiple time points. Sedation occurred within 2 to 4 min following ketamine administration and lasted 20 to 33 min. Clinical effects of ataxia, hypoactivity and sitting posture were present up to 2 hrs post-administration. Monkeys were fully recovered by 4 hrs post-administration. Food intake was decreased on the day of ketamine administration. There were no ketamine-related effects on hematology, coagulation and platelet aggregation parameters measured at 10 min, 24 hrs and 4 and 9 days post-administration. A minimal decrease in serum potassium concentrations (3.2-3.9 mEq/L *vs.* pretest values of 3.8-5.2 mEq/L) occurred in all ketamine administered monkeys at 10 min post-administration. Urine pH was minimally to mildly lower (6.0-7.5 *vs.* pretest values of 8.5) 24 hrs post-administration compared to control. These data indicate that a 10 mg/kg dose of ketamine is suitable for temporary chemical restraint of cynomolgus monkeys. However, evaluation of clinical pathology endpoints should be initiated no earlier than 24 hrs post-ketamine administration based on the minimal limited changes that occurred within 24 hrs of ketamine administration.

1281 INFRARED THERMOMETRY FOR REMOTE BODY TEMPERATURE MEASUREMENT IN THE CYNOMOLGUS MONKEY.

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Rectal thermometry is commonly used in toxicity studies for the determination of body temperature in nonhuman primates. This approach necessitates capture and handling of the animal. We, therefore, investigated the suitability of remote infrared thermometry for suface body temperature measurement in an established nonhuman primate toxicology model, the Cynomolgus monkey (Macaca fascicularis).Rectal (Gerathermâplus) and infrared (Raynger RMX4PD, Raytek) determinations were performed in eight male and female animals. Infrared measurements were conducted on caged animals and were recorded for the forehead and, among male animals, also for the testis. For all animals, data were collected for both techniques twice daily (8 am and 12 am) on 9 occasions at least one day apart.Baseline body temperature by the rectal approach in male and female animals was 38.7±0.5 °C. Forehead temperature was 31.0±0.8 °C in male and female animals. Testis surface temperature was 33.5±0.7 °C and was similar at 8 am and 12 am. Results obtained for male and female animals were comparable for both techniques. Also, the data measured were nearly identical for the 9 occasions and the two timpoints during the day.Our observations suggest that infrared temperature assessment is not suitable for determination of core body temperature. However, infrared measurements were surprisingly stable over daytime and over several days. Hence, the infrared approach is suitable for monitoring body temperature *via* surface temperature without the need of capture and handling of experimental animals.

1282 CIRCADIAN RHYTHM OF ACTH AND SERUM CORTISOL IN CONSCIOUS MONKEYS USING AN AUTOMATIC SAMPLING METHOD.

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In animals, severe stress from restraint for blood sampling can cause marked changes in biological parameters such as hormone levels. In this study, ACTH and serum cortisol levels were measured in blood sampled from conscious, unrestrained monkeys using an automatic blood sampling apparatus, to determine if the sampling method affected circadian variations. Methods: Eight male cynomolgus monkeys (BW 4 - 6 kg) were used. A polyurethane catheter for blood sampling was aseptically inserted into the femoral vein under anesthesia, and connected to a fluid swivel by a stainless tether. The swivel was connected to an automatic blood sampling apparatus by a polyurethane tube. Blood (1.5 ml) was automatically drawn every four hours and stored in a chamber (4°C). Samples were assayed for ACTH and serum cortisol levels by radioimmunoassay. Results and conclusion: Blood was sampled starting at three weeks after surgery for catheter insertion, ACTH and serum cortisol showed stable values from four weeks after surgery. ACTH levels were 13 - 18 pg/ml (daytime) and 7 - 10 pg/ml (nighttime) from four weeks after surgery, while serum cortisol levels were approximately 30 µg/ml (daytime) and 10 - 20 µg/ml (nighttime). Circadian rhythms were observed in both ACTH and serum cortisol levels, with high values during the day and low values at night. Furthermore, serum cortisol levels increased markedly according to the load of ACTH (5 µg/kg). These results demonstrate that the measurement of stable and exact ACTH and serum cortisol concentrations in monkeys is possible using an automatic blood sampling apparatus.

1283 A MODEL OF CISPLATIN-INDUCED RENAL IMPAIRMENT IN THE CYNOMOLGUS MONKEY.

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Preclinical toxicity studies for human protein drug candidates are generally conducted in normal, healthy non-human primates, which may not be representative of the target patient population. Many individuals in the target patient population may have a compromised organ system due to the existing disease-state or previous drug exposure, such as chemotherapy. While the drug candidate alone may not induce a particular toxicity, it could potentially exacerbate a pre-existing injury. For this reason, a non-human primate model of renal impairment can be a valuable tool to evaluate potential exacerbation of pre-existing renal injury by the drug candidate. This study was conducted to establish a model of mild renal-impairment in the cynomolgus monkey using clinically relevant doses of the highly nephrotoxic chemotherapeutic agent cisplatin. Adult male monkeys were administered 35 or 58 mg/m^2 cisplatin on Days 1 and 22 *via* intravenous infusion, and all animals were evaluated for changes in clinical signs, body weight, and clinical pathology indices. In order to characterize the time course of cisplatin-induced nephrotoxicity, serum chemistry parameters were evaluated at various timepoints throughout the study and two animals from each dose group were euthanized on Days 7, 21, and 42 for microscopic analysis of kidney tissue. Mild elevations in serum BUN and creatinine levels were observed in 1/6 animals in the low-dose group, whereas moderate to marked elevations were noted 3/6 animals in the high-dose group. While renal tubular necrosis ranging from minimal to marked was noted in individual animals in both dose groups at all timepoints, the incidence and severity of the renal injury was greater in the high-dose group. This study demonstrates that administration of clinically relevant doses of cisplatin results in renal toxicity in the monkey and that the nephrotoxic effects of cisplatin vary within each dose level, with a higher incidence and more severe changes occurring at the higher dose.

1284 THE USE OF THE MINI-PIG IN BIOMEDICAL RESEARCH.

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The popularity of the mini-pig in pharmacokinetic and safety evaluation studies has increased rapidly over recent years. A better appreciation of the similarities between man and the mini-pig together with increased concerns over the use of nonhuman primates are two of the principal reasons for this increase. To ensure that other researchers are aware of the potential advantages of this species presentation of background information is essential. Summary data relating to frequently measured variables including electrocardiography, clinical pathology and organ weights will be presented. The use of the mini-pig in the development of dermal products, and in the investigation of reproduction toxicity will be illustrated with data from completed studies. In addition, a number of disease models have been developed to specifically address the use of the mini-pig in Parkinson's disease, atherosclerosis and diabetes research. Data from completed studies showing the induction of symptoms of Parkinson's disease following the adminstration of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) and the successful use of this species in this type of research will be described.

1285 A CROSSOVER DESIGN FOR TELEMETRIC CARDIOVASCULAR ASSESSMENT IN UNRESTRAINED CONSCIOUS DOGS.

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Telemetry studies are part of the cardiovascular assessment of drugs, but their design has still to be optimized for appropriate analysis of repeated measurements, repeated dosings, compound comparison over successive dosing periods, in a limited number of subjects, and residual influence of treatments (carryover). In a crossover study, six beagle dogs were surgically implanted with a pressure sensor in the femoral artery for continuous recording of blood pressure (BP) and heart rate (HR), and two subcutaneous electrodes for lead II-electrocardiograms. The dogs were randomly allocated to 6 sequences each of three 5-day dosing periods with placebo, erythromycin (100 mg/kg/d sc) or sotalol (320 mg/dog/d po), the dosing periods being separated by one-week washout periods. The six sequences differed according to the six possible combinations. Evaluations included systolic and diastolic BP, HR and rhythm, PR and QT intervals and QRS complex duration. The QT interval was also adjusted for HR changes according to Bazett or Fredericia formula or using control values as a covariate. A specific statistical analysis was applied to the mini-

mum and maximum values, derived variables such as the mean daily sums ("area under the curve") and the slope and the intercept of QT=f(HR). Carryover effects and time-related effects (i.e., differences between dosing days and between dosing periods) were evaluated. Sotalol statistically significantly decreased HR and BP and significantly prolonged QT and QTc. Erythromycin resulted in increased HR, likely secondary to the local inflammatory reaction, which precluded detection of QT prolongation. The test system was stable over time. The few carryover effects suggest that washout periods have to be adjusted according to the kinetics of the compounds. This design with a limited number of animals and repeated dosing to allow detection of time-related effects and comparison of compounds is considered suitable for the nonclinical cardiovascular assessment of drugs.

1286 DEVELOPMENT AND VALIDATION OF A CANINE FUNCTIONAL OBSERVATIONAL BATTERY (FOB) FOR USE IN TOXICITY STUDIES.

J. A. Wasielewski, J. C. White, P. E. Newton, R. J. Briscoe and T. J. Baird. *Pharmacology, MPI Research, Mattawan, MI.*

Systematic observational evaluations for potential test article related acute and chronic neurofunctional effects are often standard components of general toxicity studies conducted on newly-developed pharmacologic and chemical entities, such as food additives, therapeutic drugs, and industrial/agricultural agents. The beagle dog is a frequently employed non-rodent species in regulatory toxicology studies, and in a considerable amount of discovery/efficacy work. However, the vast majority of standardized neurobehavioral evaluations are performed in rodents (Irwin, 1968; Gad, 1982), and progress towards a validated FOB in canines (i.e., Haggerty, 1991) has been slow. Prior investigations have established the susceptibility of dogs, like rodents, to a variety of neurotoxic agents. Still more literature establishes the dog as a sensitive model for detecting and characterizing the adverse neurofunctional effects of pharmaceuticals. The need for, and utility of, a robust and yet sensitive screening methodology for detecting and providing initial characterization of such neurotoxic and neurofunctional effects is clear, and has now been developed and validated in the beagle dog. Initial validation of this 39-item FOB was performed using four common pharmacologic positive control compounds: haliperidol (3.0 mg/kg, s.c.), ethanol (2.0 g/kg, p.o.), pilocarpine (2.0 mg/kg, s.c.) and amphetamine (2.0 mg/kg, s.c.). Results indicate that this observational battery is sensitive to the known acute pharmacological effects of these agents. Additional work is planned to further establish the capacity of this FOB to detect and characterize neurobehavioral pathology associated with chronic exposure to known neurotoxicants.

1287 PERFORMANCE VALIDATION AND DEMONSTRATION OF THE SENSITIVITY TO DETECT DRUG INDUCED ALTERATIONS USING A LOCOMOTOR ACTIVITY SYSTEM FOR LARGE ANIMALS.

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Neurotoxicity regulatory guidelines require that automated systems which quantify activity be evaluated to ensure the reliability of operation across devices and that the system be able to detect chemically induced changes in experimental endpoints. This study was designed to evaluate the performance and sensitivity of a system (Hamilton-Kinder MotorMonitor Activity System) to measure locomotor activity in large animals. The performance validation consisted of eight sessions for each of four enclosures; two each day; one in the am and one in the pm for four consecutive days. At random intervals throughout the 1-hour session a remote control was used to steer an object in each enclosure. The total rest time of each performance session for the object was calculated manually and compared to the total rest time recorded by the system software. To demonstrate the sensitivity of the system to detect drug-induced changes in motor activity, 12 adult male Beagles were used in a Latin Square design in which each animal served as its own saline control and received first d-amphetamine (0.1, 0.3 and 1 mg/kg i.m.) and then chlorpromazine (1, 3 and 10 mg/kg i.m.) during the course of the study. Basic movements (fine + ambulatory counts) were recorded in each 60-minute test session which consisted of twelve 5-minute epochs. The results for the performance evaluation indicated a mean difference of < 2 seconds between the manual and software calculated total rest times for each enclosure over four consecutive days, which met the acceptance criteria of ± 5 seconds. Administration of d-amphetamine resulted in detectable dose-related increases of 50%, 66% and 79% in basic movements above control values, while chlorpromazine induced detectable dose-dependent decreases of 25%, 53% and 70% below controls for basic movements. These results demonstrate that the performance of this activity system is reliable and can detect chemically induced changes in locomotor activity for Beagles.

1288 CONFIRMATION OF BLOOD IN FECES USING HEMATEST® REAGENT TABLETS: COMPARATIVE EVALUATION WITH CANINES, NON-HUMAN-PRIMATES, AND RATS.

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Hematest® Reagent Tablets, marketed for the detection of occult blood in human feces, have been used to confirm the presence of blood in laboratory animal fecal material. Discrepancy between visual observation and assay results forced into question the utility of the test in laboratory animals. The purpose of this study was to determine whether Hematest® Reagent Tablets provide useful information for confirming the presence of blood in laboratory animal feces. Fecal observations were recorded from 5 canines and non-human primates (NHP) per sex and from 5 cages of rats per sex (2 per cage) for 10 days. Four random samples per animal or cage were tested per day with the Hematest® Reagent Tablets. All fecal samples were visually observed for the presence of red pigment and those containing red pigment were tested in lieu of a random sample. Of the 396 canine samples collected, approximately 4% were visually noted to contain red pigment; 71% of those samples observed with red pigment tested positive for blood in feces. Of the total number of samples observed with or without red pigment, 60% tested positive for the presence of blood with Hematest® tablets. In NHP, 8% of all fecal samples observed contained red pigment, of which 91% subsequently tested positive for blood in feces. Hematest® results were positive in 46% of the total NHP fecal samples collected. In rats, no samples observed during the 10 days were visually noted to contain red pigment, however, 89% of all fecal samples collected tested positive for occult blood. These findings indicate that in the species examined and manner used, Hematest® Reagent Tablet assay results do not agree with the observations of red pigment (or lack thereof) in feces. Because of the high incidence of positive results in naïve animals, the test may be inappropriate and misleading when used in laboratory animals to confirm a visual observation of red pigment as blood in feces.

1289 FECAL BILE ACID EXCRETION AND PROFILE IN THE BEAGLE DOG CHARACTERIZATION ACCORDING TO SEX AND AGE.

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Beagle dogs are commonly used as a nonclinical model for the safety assessment of industrial chemicals, pesticides and human pharmaceuticals. In general each laboratory maintains a historical data base for routine parameters including hematology and clinical chemistry values. However, similar data are limited or not available for non-routine tests such as fecal bile acid (FBA) excretion and profile. Thus, as a reference, FBA excretion and profile were determined in male and female beagle dogs at ages ranging from 6 to 18 months. Feces were collected over a 48-hour period and were processed and evaluated enzymatically for the concentrations of 3α-hydroxy bile acids (which account for approximately 85% of total fecal bile acids in dogs). These samples were further analyzed by LC/MS to provide concentrations of specific α-hydroxy bile acids -cholic acid (cholic), deoxycholic acid (deoxy), chenodeoxycholic acid (cheno), and lithocholic acid (litho). The results obtained are presented in the table below. Fecal bile acid excretion decreased with age in males. Deoxycholic acid was the predominate bile acid in feces. There were no major changes in the proportions of the major α-hydroxy bile acids between sexes and among the different age groups. However, the ratio of Deoxycholic:Cholic acid was greater in females than males indicating greater intestinal bacterial dehydroxylation in females.

MALE					
Age (months)	**Fecal Bile Acids (μmoles/kg/day)**	**Cholic * (%)**	**Deoxy* (%)**	**Cheno* (%)**	**Litho* (%)**
6	45.0	5.6	76	2.4	15.9
12	30.8	9.6	75.1	2.3	13.0
18	19.9	7.6	73.5	2.2	16.7
FEMALE					
6	40.3	6.5	84.8	1.6	13.8
12	36.7	9.7	78.3	1.7	10.2
18	29.0	4.5	81.3	1.3	12.9
*The proportion or percentage of each of the bile acids was calculated by dividing the concentration of each bile acid by the total concentration of the four bile acids examined					

1290 A COMPARISON OF FECAL BILE ACID EXCRETIONS IN CD, WISTAR, LONG EVANS, HSD:SD, AND F344 FEMALE RATS.

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Rodent suppliers maintain and publish hematology and clinical chemistry values for routine parameters. However, similar data are not available for non-routine tests such as fecal bile acid (FBA) excretion. Therefore, to provide this information for reference, and to compare strain variations, fecal bile acid concentrations were determined for five different strains of female rats: Crl:CD®(SD)IGS BR and Crl:(WI)BR (Charles River, Portage, MI), Crl;(LE)BR, (Charles River, Rawleigh, NC), and Hsd:SD and F344/NHsd (Harlan Sprague-Dawley, Indianapolis, IN). The age of the females ranged from 8 to 11 weeks. Feces were collected over a 72-hour period and were processed and evaluated enzymatically for the concentrations of 3α-hydroxy bile acids (which account for approximately 70% of total fecal bile acids). These samples were further analyzed by HPLC to determine the concentrations of specific bile acids: cholic acid (cholic), deoxycholic acid (deoxy), chenodeoxycholic acid (cheno), and lithocholic acid (litho). The results obtained are presented in the table below. Fecal bile acid excretions ranged from 67.9 µmol/kg/day for the HSD rats to 104.6 µmol/kg/day for the F344 rats. F344 rats had higher concentrations of deoxycholic acid than the other strains. In general, concentrations of cholic acid and lithocholic acid were similar among the strains. Chenodeoxycholic acid was usually present at concentrations below the level of sensitivity of the assay. Concentrations are presented in µmol/kg/day

Strain	FBA	Cholic	Deoxy	Cheno	Litho
CD	91.1	0.867	3.804	0	2.237
WI	67.9	0.404	2.797	0	2.515
LE	97.1	2.087	4.293	0.354	3.917
HSD	76..6	0.282	3.366	0.010	2.003
F344	104.6	0.506	6.753	0	3.035

1291 A COMPARISON OF THE IN-LIFE PARAMETERS AND TUMOUR DATA FROM TEN GANG-HOUSED DIETARY TUMORIGENICITY STUDIES USING THE SPRAGUE-DAWLEY INTERNATIONAL GENETIC STANDARD OR HAN WISTAR STRAINS OF RAT.

W. N. Hooks and S. M. Harling. *Toxicology/Pathology, Huntingdon Life Sciences, Huntingdon, United Kingdom.* Sponsor: C. Atterwill.

The in-life and tumour data were reviewed from the control groups of ten gang-housed dietary tumorigenicity studies completed over 1994 to 1999. Five studies using the Sprague-Dawley International Genetic Standard rat (SD IGS) from Charles River, UK were compared with five studies using the Han Wistar rat from Bioresearch Laboratories, Switzerland. Low protein maintenance diet was used in these studies. The comparisons have shown that the percentage mortality at 2 years for the Han Wistar rat studies (18% in males and 26% in females) was considerably lower than that of the SD IGS rat studies (57% in males and 66% in females). The bodyweight and food consumption data obtained for the Han Wistar rat were also considerably lower in comparison with the SD IGS rat. The most prevalent tumours in both groups were pituitary adenoma in both sexes and mammary tumours in females (also the major pathological factors contributory to death). However, the incidence of these tumours was lower in the Han Wistar rat group in both sexes. Other notable differences between the groups were a higher incidence of subcutaneous fibroma and adrenal phaeochromocytoma in SD IGS male rats and lymph node haemangioma in Han Wistar male rats. In conclusion, the Han Wistar rat has a satisfactory tumour profile and, when compared with the SD IGS rat, a high survival rate. Additionally, on the basis of regulatory requirements to reach 2 years with an adequate number of survivors the Han Wistar rat would be a suitable strain to use without recourse to diet optimisation methods.

1292 EVALUATION OF SELECTED PSYCHOACTIVE DRUGS IN THE RAT USING A MODIFIED IRWIN TEST.

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The characterization of the general pharmacology of drugs by observational techniques in mice is a widely-used approach in the pharmaceutical industry to profile the properties of newly-synthesized agents and to evaluate their safety prior to clinical testing. A systematic study to characterize the effects of some selected psychoactive drugs has been carried out in the rat using a modification of the original Irwin procedure. Groups of 6 male Sprague-Dawley rats were orally dosed with vehicle (1% methylcellulose), chlorpromazine (10-50 mg/kg), amphetamine (2-10 mg/kg), diazepam (10-50 mg/kg) or morphine (10-50 mg/kg). Each rat was then systematically evaluated using a procedure in which previously-defined parameters were scored (0-8) according to their intensity. Parameters already present were scored "4" with potentiation or depression scored higher (4-8) or lower (4-0), respectively. Rats dosed with chlorpromazine showed catalepsy, decreased alertness, decreased sensory responses, hypothermia and ptosis. Rats dosed with amphetamine displayed behavioural arousal with increased locomotor activity, restlessness, increased sensory responses, piloerection and stereotyped behaviour. Rats dosed with diazepam displayed behavioural passivity with mild catalepsy, decreased alertness, decreased grooming, some decrease in sensory responses with some impairment of the righting reflex. Rats dosed with morphine displayed increased alertness, increased grooming and increased locomotor activity, exopthalmos, decreased response to pain and compulsive licking. The general observations were consistent with varying levels of depression or stimulation of the central nervous system together with specific and characteristic signs for the drugs tested and it is concluded that observational testing in the rat using a modified Irwin procedure provides a means of discriminating drugs on the basis of changes in behaviour and identifying their safety.

1293 APPLICATION OF UNRESTRAINED WHOLE BODY BIAS FLOW PLETHYSMOGRAPHY TO MEASURE RESPIRATORY PARAMETERS IN THE CONSCIOUS RAT.

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It is essential that potential undesirable effects on the respiratory system, of new pharmaceutical, veterinary or agricultural products, are properly evaluated prior to exposure to humans. In this context, the revised ICH Guidelines on Safety Pharmacology Studies for Human Pharmaceuticals (S7A) identify assessment of *in vivo* effects on the respiratory system, as part of the Safety Pharmacology core battery. To meet the S7A guidelines and as part of our safety pharmacology R&D programme, the Department of Pharmacology has developed procedures for the measurement of a number of respiratory parameters in conscious, freely moving rats. The equipment (Buxco Electronics) comprises whole body plethysmographs connected to a data acquisition computer enabling real time analysis (using Biosystem XA software). This allows the determination of a number of respiratory parameters including respiration rate, tidal volume and minute volume. Preliminary baseline studies showed that there were no statistically significant differences in respiratory parameters between Sprague-Dawley and Wistar rats, sex or time of day of recording although not surprisingly, there was evidence of habituation with repeated or prolonged exposure. To demonstrate ability of the system to detect drug effects on respiratory parameters, studies were conducted using morphine sulphate and theophylline, administered as single dosages either by oral gavage or intravenous injection, to male and female Wistar rats. As expected, relative to vehicle-treated animals, morphine depressed respiration whereas theophylline increased respiration rate with consequential increases in minute volume. These drug results confirm the ability of whole body plethysmography to detect potential side effects of substances on the respiratory system in freely moving rats. The methodology ideally meets the ICH guidelines for an *in vivo* core safety pharmacology study using conscious animals under the preferred conditions of nonrestraint.

1294 IV LOCAL TOLERANCE STUDIES: HOW TO REDUCE THE NUMBER OF ANIMALS?

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For oral compounds (iv bioavailability study in man) local tolerance is usually assessed in rat. For iv compounds, general toxicology studies can be used to assess this. For compounds infused in non-clinical trials *via* central veins, local tolerance may have to be assessed in a peripheral vein: first choice again is rat. Due to too long study duration and small size of rodent veins, other species could be preferred. Generally no bolus injections are performed but slow injections, or short or continuous infusions. Centrally a vena cava is used for continuous infusion. The anterior one should be avoided for local tolerance, because it is difficult to be sure that the tip of catheter was in vein and not in heart. Different injection sites can be used superficially. For rats the tail vein is appropriate. For rabbits and minipigs a marginal ear vein is generally used. Other superficial injection sites could be used to check the risk of misdosing. Several concentrations may have to be tested and placebo and ideally saline control groups should be added. The total number of animals can thus be quite high. Especially if several veins have to be used, several necropsy occasions are scheduled and/or further groups have to be added, taking into considera-

tion first findings (with first tested concentrations). We propose different ways to reduce this number. 1/ Several veins from the same animal (e.g., one central and four superficial veins on different days) could be used, allowing evaluation of local tolerance at different times after treatment. 2/ Animals implanted and dosed in different weeks (however needed for continuously infused animals, e.g., one week per concentration), allowing easier and better concentration selection and avoiding useless concentrations. 3/ Some investigations could be performed during pilot escalating dose studies (e.g., on different days before necropsy): visual evaluation and grading of the external aspect of the superficial injection sites, and echography or MRI of superficial as well as central vessels.

1295 A PRIMARY SKIN IRRITATION STUDY OF ELECTROTRANSPORT SYSTEMS ON HAIRLESS GUINEA PIGS AND RABBITS.

A. E. Chester and P. Quigley. *NonClinical R&D, ALZA, Mountain View, CA.*

The objective of this study was to evaluate the potential degree of dermal inflammation produced by three different E-TRANS® (placebo) systems containing cetylpyridinium chloride (CPC)(0.08% or 0.2%) in the cathode hydrogel and one of two adhesives to adhere the systems to the skin. Each system had an anode and cathode. All of the systems had the same anode hydrogel formulation. Systems were applied to the intact dorsal skin of 12 hairless guinea pigs (1 or 2 systems/guinea pig) and 6 New Zealand White (NZW) rabbits (3 systems/rabbit). The total wearing time was approximately 13.3 hours. The direct current density was approximately 71.4 $\mu A/cm^2$ (based on a hydrogel area of 1.4 cm^2 and a total current of approximately 100 microamps) at both the anode and cathode. Application sites were scored for erythema and eschar formation and edema (modified Draize method) 0.5, 24, and 48 hours after system removal, and Primary Irritation Indexes were calculated. No treatment-related changes occurred in body weights or clinical conditions. No mortality occurred. The Primary Irritation Indices are presented below. Histopathology results supported the mild irritation observed. In conclusion, the cathode hydrogels containing 0.08% or 0.2% CPC were mild irritants in guinea pigs and rabbits. The anode hydrogel was a mild irritant in guinea pigs and a non-to mild irritant in rabbits.

	Hairless Guinea Pig		NZW Rabbit	
Cathode Formulation	Anode	Cathode	Anode	Cathode
0.08% CPC/Adhesive 1	1.3 (mild; n=5)	1.6 (mild; n=5)	0.0 (none; n=6)	1.4 (mild; n=6)
0.2% CPC/Adhesive 1	0.9 (mild; n=6)	2.0 (mild; n=6)	0.3 (none to negligible; n=3)	1.8 (mild; n=3)
0.2%CPC/Adhesive 2	1.3 (mild; n=6)	2.0 (mild; n=6)	0.6 (mild; n=4)	1.6 (mild; n=4)

1296 ASSESSMENT OF RETINAL DEGENERATION IN OUTBRED ALBINO MICE.

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Complete evaluations of a drug's safety necessarily include an ophthalmic assessment. Various stocks of mice are typically used in nonclinical safety studies. However, despite the genetic diversity that breeders attempt to maintain in outbred populations, certain genetic constitutions develop that provide sensitivities of which both breeders and toxicologists are unaware. Increased sensitivity to light in a laboratory animal can confound the interpretation of ophthalmic data generated from a particular study. Retinal degeneration (RD) was observed to occur in outbred albino mice (HsdICR.CD1). Such a pathologic manifestation has been reported to be associated with an autosomal recessive gene rd, retinal degeneration. In an effort to evaluate the genetic basis for the observation of RD in HsdICR.CD1 mice, a study was conducted comparing the light sensitivity of the following stocks/strains of mice: Hsd:ICR(CD-1); Hsd:NSA(CF-1); HsdWin:CFW-1; Crl:CD-1(ICR)BR; Crl:CF-1BR; Crl:CFW(SW)BR; Tac:Icr:Ha(ICR); Tac(SW); FVB/NCrlBR; and C57BL/6J-$Tyr^{c-2J/+}$. FVB/NCrlBR was used as a positive control since it has been established that these animals carry an rd gene. C57BL/6J-$Tyr^{c-2J/+}$ was used as a negative control since these animals have been reported to be resistant to retinal degeneration. Mice were observed over a twelve-week period of time. Average room and in-cage illumination were determined. Hematology and serum clinical chemistry revealed no abnormal findings. Histopathologic evaluation revealed clear differences. Affected stocks/strains did not exhibit sex-related differences, except for Hsd:ICR(CD-1) mice, in which retinal atrophy was almost twice as common in males as females. Incidences of retinal atrophy for the various stock/strains were: Hsd:ICR(CD-1) (43.3%); Crl:CF-1BR (3.0%); Crl:CFW(SW)BR (98.3%); Tac:Icr:Ha(ICR) (75%); Tac(SW) (80%); and FVB/NCrlBR (100%). No retinal atrophy was observed in the following strains: Hsd:NSA(CF-1); HsdWin:CFW-1; Crl:CD-1(ICR)BR; and C57BL/6J-$Tyr^{c-2J/+}$.

1297 BIOPHOTONIC IMAGING IN HO-LUC TRANSGENIC MICE: REAL-TIME DEMONSTRATION OF GENDER SPECIFIC CHLOROFORM INDUCED RENAL TOXICITY.

L. R. Weir, E. Schenk, J. Meakin, R. Driver, K. Rush, F. McClure, J. Fuller, M. York and A. M. Lynch. *Safety Assessment, GlaxoSmithKline, Welwyn, United Kingdom.* Sponsor: P. Trennery.

The metabolism of luciferin in mice transgenic for luciferase (HO-luc) produces light that may be detected *'trans-vivo'* by an intensified CCD camera (biophotonics). Thus the generation of transgenic promoter-luciferase animals for genes regulated by specific toxic processes, coupled with real-time evaluation of site-specific gene expression may provide novel, non-invasive *in vivo* biomarkers which are predictive of developing toxicity. As part of a programme to evaluate the potential of biophotonics for predictive toxicology we have conducted a series of studies in HO-luc mice, transgenic for the murine heme oxygenase-1 promoter (Xenogen, CA, USA). Chloroform induced renal toxicity shows a marked gender difference in mice, with males being much more sensitive than females due to the expression of CYP2E1 in male mouse kidney. CYP2E1 mediated oxidative metabolism converts chloroform to phosgene and results in covalent binding to proteins and depletion of glutathione. However, when female mice are pre-treated with testosterone the expression of renal CYP2E1 removes the gender difference and the kidneys become susceptible to chloroform toxicity. As part of the evaluation of HO-luc Tg mice, male and female animals were treated with chloroform (200 mg/kg, p.o., daily for 5 days) and imaged 2 and 6 hours after dosing. During a two-day washout period, female animals were treated daily with testosterone prior to repeat administration of chloroform for a further 5 days. Comparison of the *in vivo* response of the luciferase reporter with markers of toxicity measured *ex vivo* (differential gene expression of antioxidant system response genes, clinical chemistry and pathology) confirms the gender specific difference in chloroform renal toxicity in HO-luc Tg mice, and correlates with the expression of the endogenous HO-1 gene. These studies demonstrate the capacity of biophotonics for real-time site-specific gene expression, which may be predictive of developing toxicity.

1298 INTERPRETING TOXICITY STUDIES; RECOGNISING EFFECTS AND DIFFERENTIATING BETWEEN ADVERSE AND NON-ADVERSE EFFECTS.

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It is difficult to find criteria that can be used to guide consistent interpretation of toxicity studies. A two step process is outlined to address this issue. Differences from control values are initially evaluated in order to decide if they are treatment related or if they are chance deviations. In the second step, only those differences judged to be effects are further evaluated in order to discriminate between those that are adverse and those that are not. In differentiating an effect from a chance finding, criteria used to aid evaluation are: Dose-Response, consideration of spurious measurements, the precision of the measurement under evaluation, ranges of natural variation and the overall biological plausibility of the observation. In discriminating between the adverse and the non-adverse effect it is important to consider: whether the effect is an adaptive response, whether it is transient, the magnitude of the effect, its association with effects in other related endpoints, whether it is a precursor to a more significant effect, whether it has an effect on the overall function of the organism, whether it is a specific effect on an organ or organ system or secondary to general toxicity or whether the effect is a predictable consequence of the experimental model. In interpreting complex studies it is recognised that a weight of the evidence approach, combining the criteria outlined above to reach an overall judgement, is the optimal way of applying the process. It is believed that the use of such a scheme will help to improve the consistency of study interpretation that is the foundation of hazard identification and risk assessment.

1299 APPLICATION OF *IN SILICO* APPROACHES TO TOXICITY PREDICTION.

A. C. White, R. A. Mueller and A. G. Wilson. *Preclinical Development, Pharmacia Corporation, St. Louis, MO.*

In recent years there has been an increasing requirement for the early selection of lead drug candidates as a way to improve the efficiency of the drug discovery process. This has led to the emergence of highthroughput screening assays for

ADME and toxicity prediction. A rapidly developing adjunct to this approach for drug discovery is the use of computer based (*in silico*) prediction tools. In this presentation we will describe the results of a validation study of over 2400 structures analyzed by predictive systems (TOPKAT, DEREK, CASETOX) and compare their accuracy to predict toxicity for a series of proprietary and commercial molecules. Statistical analysis of the results showed the accuracy of individual programs for novel structures to be in the range of 65-75%. When we used the predictive systems in combination the accuracy increased to greater than 90%. We have developed a decision tree approach to support the use of these programs for decision-making that will also be discussed.

1300 SAFETY EVALUATION OF PHENOL AND *M*-CRESOL AS INACTIVE INGREDIENTS IN SUBCUTANEOUS DOSAGE FORMULATIONS.

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Phenol and *m*-cresol exhibit a wide range of overlapping commercial applications as demonstrated by their usage in the pharmaceutical, chemical, and agricultural industries. In pharmaceutical applications, their primary functional role is the antimicrobrial preservation of drugs formulated for intramuscular, intradermal, and subcutaneous injections. Extensive use of these compounds in approved subcutaneous dosage forms is well documented in the US Food and Drug Administration's *Inactive Ingredients Guide* at levels up to 0.5% for phenol and 0.31% for *m*-cresol. In order to understand more fully any potential risks associated with the use of these compounds, the comparative safety of phenol and *m*-cresol was investigated. In acute subcutaneous toxicity studies performed in mice, rats, guinea pigs, and rabbits, the LD_{50} values for phenol and *m*-cresol were comparable and in excess of 300 mg/kg. Similar toxicological profiles (reduced water consumption, body weight loss, and nephropathy) were observed in 90-day rodent toxicity studies. In F344 rats treated with 0 to 780 mg/kg/day phenol in drinking water for 90 days, the lowest-observed-adverse-effect level (LOAEL), based on decreased water intake and body weight gain, was 780 mg/kg/day. A LOAEL, also based on weight gain reduction, was observed at 150 mg/kg/day in Sprague-Dawley rats administered *m*-cresol (0 to 450 mg/kg/day) by oral gavage for 90 days. It is concluded that the use of either of these compounds in subcutaneous dosage formulations is strongly supported by the similarity of the results in available animal data, and preference for one agent over the other should be based upon other considerations.

1301 A 3-MONTH DIETARY TOXICITY STUDY IN RATS WITH POLYVINYL ALCOHOL.

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Polyvinyl Alcohol (PVA) is a water-soluble synthetic polymer prepared from the polymerization of vinyl acetate and the subsequent partial hydrolysis of polyvinyl acetate. PVA is used as a polymer for film coating pharmaceutical and dietary supplement tablets where moisture barrier/protection properties are required. A special grade of PVA was used in this study that is produced to meet global pharmaceutical standards using good manufacturing practices (GMP's). The PVA grade is characterized by compendial and proprietary specifications. The impurities are strictly monitored by process controls, stringent specifications and analytical methods developed by the manufacturer. The potential systemic and neurotoxicity of PVA was assessed when fed in the diet to Sprague-Dawley rats (20/sex/group) for 90 days at dose levels of 2000, 3500 and 5000 mg/kg/day. Control rats received untreated standard laboratory diet. Assessments included clinical observations, ophthalmology, body weight and food consumption, hematology, coagulation, clinical chemistry, urinalyses and motor activity and functional observational battery evaluations. After 3 months of treatment, selected organs were weighed, and complete macroscopic as well as histopathological and neuropathological evaluations were conducted. The only test article-related finding observed during the study was unformed stool with brown/black anogenital staining in rats fed 3500 and 5000 mg/kg/day. This finding was attributed to the high levels of test article being consumed and subsequently excreted in the stool. It was not accompanied by changes seen macroscopically or microscopically in these rats. No test article-related changes were seen in mortality, ophthalmology, bodyweight and food consumption data, hematology, clinical chemistry, urinalysis data, functional observational assessments, motor activity, organ weight data and macroscopic and microscopic examinations. Dietary concentrations of 2000, 3500 and 5000 mg/kg/day of PVA to Sprague-Dawley rats for up to 90 days did not result in any adverse, toxicological effects.

1302 13-WEEK GAVAGE TOXICITY STUDY OF 5-(HYDROXYMETHYL)-2-FURFURAL (HMF) USING F344 RATS AND B6C3F1 MICE.

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HMF, a furan compound formed during the thermal decomposition of hexose sugars and carbohydrates, is ubiquitous in the human diet and a contaminant of parenteral solutions of glucose and fructose sterilized by heating or autoclaving. F344/N rats and B6C3F1 mice (10 animals/sex/group) were given HMF by gavage at doses of 47 (mice only), 94, 188, 375, 750, or 1500 (rats only) mg/kg, 5 days/week for 13 weeks. Controls received the vehicle only (deionized water). Survival decreased for the 1500 mg/kg group male (9/10) and female (7/10) rats. Group mean body weight decreased 14 percent (relative to control) for the male 750 mg/kg group mice. Rat urine volume increased (1500 mg/kg) and urine creatinine decreased (≧375 mg/kg, m; ≧750 mg/kg, f). There were no HMF-related changes in clinical observations and clinical pathology. Mouse kidney weights decreased and microscopic findings were limited to large clear cytoplasmic vacuoles in the proximal tubular epithelial cells (≧188 mg/kg, m). Gross and microscopic findings were similar to control for the rats. (Supported by Contract No. N01-ES-15320.)

1303 ACUTE AND CHRONIC TOXICITY OF BITUMEN-SALT MASS END-PRODUCTS FROM THE DESTRUCTION OF RUSSIAN SARIN, SOMAN AND VX.

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In support of Cooperative Threat Reduction, the Russian Federation (RF) (GosNIIOKhT) participated in joint studies with the U. S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) to investigate the toxicity of materials leached from bituminous salt masses (BSM) containing by-products from a chemical demilitarization process developed by the RF. These studies determined hazard categories for the BSMs and evaluated the effects of long-term exposure to leachates from the BSMs in animals. Acute oral toxicity values (LD50) were determined in rodents for the components of BSM resulting from the destruction of Russian sarin (GB), soman (GD) and VX. Inhalation studies in rats evaluated the toxicity of volatile components resulting from heating (up to 130 °C) the BSMs, a process similar to that involved in the second phase of BSM production. Chronic toxicity was also investigated over six months in rats. Using a leaching process developed by GosNIIOKhT, aqueous leachate solutions were produced from bitumen alone, or GB-, GD- or VX-BSM. For each leachate, five groups of animals were dosed orally five days per week. Three groups were administered leachate at concentrations of 100%, 20% or 4%. Two other groups served as controls and were exposed to either leachate derived from bitumen alone, or ground water. Various physiological, biochemical and hematological indices as well as histopathology were evaluated. Results indicated no adverse effects in rats following inhalation of volatile components or chronic oral exposure to leachates from GB-, GD- or VX-BSM. Data from these experiments placed each BSM into a low hazardous toxicity category (Category IV), according to RF standards. The data is supported by toxicity studies conducted by USACHPPM.

1304 NINETY-DAY SUBCHRONIC ORAL TOXICITY OF AQUEOUS LEACHATES DERIVED FROM BITUMINOUS-SALT MASSES CONTAINING REACTION BY-PRODUCTS FROM THE NEUTRALIZATION OF U. S. OR RUSSIAN NERVE AGENTS.

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In support of Cooperative Threat Reduction activities, the U. S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) participated in joint studies with the Russian Federation (RF) to evaluate the subchronic oral toxicity of materials leached from the bituminous-salt mass (BSM) end-products of a chemical demilitarization technology developed by the RF. Subchronic oral toxicity studies were conducted by USACHPPM to address concerns that materials leached from the BSMs could pose a potential health threat to receptors in the vicinity of future storage/disposal sites in Russia. Aqueous leachate materials derived from Russian bitumen alone, BSM containing demilitarized U. S. manufactured sarin (GB), or Russian manufactured GB, soman (GD) or VX were prepared using a toxicity characterization leaching procedure (TCLP) and administered by gavage to adult male and female rats for 90 days. Dosage levels of leachate were set at 0, 4, 20, and 100%

of the test material, and were administered at 10 ml/kg body weight. Data included measurements of body weight, food consumption, serum chemistry, hematology, urinalysis, organ weights, histopathology, and immunotoxicology parameters. The results indicated that aqueous leachate materials derived from US GB-BSM, or RF GB-BSM, GD-BSM or VX-BSM caused no significant adverse health effects when administered subchronically to rats for the given test periods. This data supports and enhances the findings of additional joint toxicology studies conducted in Russia.

1305 GENOTOXICITY AND 90-DAY/DEVELOPMENTAL TOXICITY STUDIES ON AN EXPLOSIVE FORMULATION.

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Composition B Replacement 12 (PAX-21) is a new explosive formulation that is being considered for replacement of the current explosive, Composition B (Comp B). PAX-21 differs from Comp B because it contains new components, such as 2, 4-dinitroanisole (DNA) and ammonium perchlorate (AP). Genotoxicity and 90-day/developmental toxicity studies were performed to evaluate potential toxic effects of PAX-21. PAX-21, Comp B, and DNA were mutagenic to bacteria (Ames assay). However, these substances were not mutagenic in Chinese Hamster Ovary cells (AS52/XPRT assay) and did not induce micronuclei in the Swiss CD-1 mouse polychromatic erythrocyte system. Mortality resulted in rats exposed for two weeks (6 hr/d, 5d/wk) to aerosol concentrations of DNA (the most volatile component of PAX-21)≧ 545 mg/m^3. Mild signs of toxicity and non-specific minimal metaplasia of laryngeal epithelium were observed at 165 mg/m^3. Rats were dosed (gastric intubation) with 0 (control), 15, 30, or 90 mg/kg-d PAX-21 for 90 days and monitored for general toxicity, including histopathology, neurotoxicity and blood methemoglobin measurements. Partial mortality was observed at 90 mg/kg-d, liver and thyroid weight alterations with slight (adaptive) heptaocellular hypertrophy at 30 mg/kg-d, and no observable adverse effects at 15 mg/kg-d (NOAEL). In a prenatal developmental toxicity study, rats were dosed (gastric intubation) with 0, 15, 30, or 60 mg/kg-d PAX-21 on gestation days 6-19. Mortality was observed at 60 mg/kg-d, maternal signs of toxicity and slight fetal body weight alterations at 30 mg/kg-d, and a NOAEL at 15 mg/kg-d. Results from these studies were used to propose an occupational exposure level of 0.35 mg/m^3 for PAX-21. (Supported by US Army Product Manager for Mortars in collaboration with US Air Force AFRL/HEST)

1306 APPLICATION OF TOXICOLOGY IN THE DEVELOPMENT OF ENVIRONMENTALLY-FRIENDLY ELECTRONIC COUNTERMEASURES.

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Millions of thin, aluminum-coated fiberglass dipoles (i.e., "chaff"), ranging from 0.8 –7.5 cm in length, can be deployed by military aircraft and vehicles to confuse enemy radar. Chaff is stored onboard vehicles in small cartridges that consist of a piston, 0.5-1.5 million chaff fibers, a cartridge body, an endcap, and either a pyrotechnic charge or spring-loaded release mechanism that forces the piston through the cartridge body resulting in the liberation of the endcap and chaff dipoles. The accumulation of discarded styrene piston and endcaps in the environment from chaff training missions is a concern of the Department of Defense and other governmental agencies. The US Navy has embarked on a program to develop degradable chaff cartridges, pistons, and endcaps by the 2003 fiscal year. Five biodegradable materials are being considered for use. Degradability and environmental toxicity tests are helping to select the most suitable construction materials. Relative degradability of the materials is being evaluated by measuring total organic carbon (TOC) released by the materials over time in deionized water. Environmental toxicity testing of the biodegradation products from the five materials is being conducted in 2 terrestrial plant species and 7 species of aquatic organisms. Biodegradation products from each of the five biomaterials under consideration did not inhibit seed germination or affect shoot growth of Brassica rappa or Lepidium sativum significantly. Biodegredation products from 3 of the biopolymers were found to be toxic to aquatic organisms with LC50s ranging from 0.6 – 14.7 mg/l TOC. Information gained from these studies will be used for making decisions on which (if any) of the polymers will be suitable for the construction of biodegradable chaff cartridges, pistons, and endcaps. Tensile strength, ease of casting, production waste stream, and overall cost of manufacture are factors that also need to be considered.

1307 QUANTITATIVE DETERMINATION OF PETROLEUM HYDROCARBONS IN INDOOR AIR: AN ON-SITE TOOL FOR RISK ASSESSMENT.

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Many homes in the United States are heated with #2 fuel oil. In northern climates, fuel oil storage tanks are often kept in basements to avoid gelling during the winter months. During catastrophic flooding events, these tanks may rupture and release their contents. The spilled fuel oil then absorbs into the building materials (e.g. wood and concrete) and subsequently desorbs into the indoor air over time. Methods for on-site, quantitative determination of petroleum hydrocarbons in air were explored. Currently accepted methodologies (e.g. USEPA standard methods) require that air samples, regardless of sampling method, be transported to an off-site laboratory for analysis. During this transport, the possibility of analyte loss is introduced, and inaccuracies may arise in the obtained results. Accurate measurement of the concentration of airborne pollutants is essential for the determination of the health risk to individuals. On-site analyses not only reduce the risk of analyte loss, the results are also immediately available, and appropriate actions (i.e. removal of individuals present) may be taken with little or no delay. Portable Gas Chromatography (GC) equipped with an adsorbent resin (Tenax GR) trap was used for direct air sampling and chromatographic separation of gasoline, diesel fuel, aviation gas and #2 fuel oil. Analyses were also carried out with a hand-held Photoionization Detector (PID), which were compared to the GC results. The GC method showed comparable response factors for all test fuels, whereas the PID showed an increased response factor for gasoline. Both methods were linear over the tested range of concentrations, with good to fair reproducibility. A study of aging #2 fuel oil over a period of two weeks showed a fairly smooth drop of in response using GC methods, and somewhat erratic response with the PID.

1308 EVALUATION OF POTENTIAL HEALTH EFFECTS FROM INHALATION EXPOSURE TO MYCOTOXINS IN INDOOR OFFICE AND RESIDENTIAL ENVIRONMENTS.

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There have been numerous claims of human toxicity from molds in indoor office and residential environments. None of these claims include a quantitative analysis of exposure. To determine an upper limit of exposure to mycotoxins in office and residential environments, we created a simple inhalation exposure model based upon concentrations of representative mycotoxins in mold spores. The model uses: 1) the amount of mycotoxin per spore as reported in peer-reviewed literature, 2) the greatest concentration of spores which, in our experience, could credibly be present in continuously occupied space, 3) 100% retention of inhaled particles, 4) and 24 hour/day exposure duration. The maximum modeled exposures of some mycotoxins potentially found in molds that may grow indoors are: fumitremorgen A, 5.4 x 10^{-7} mg/kg; satratoxin H, 1.6 x 10^{-7} mg/kg; and trichoverrols, 5.6 x 10^{-8} mg/kg. These exposures are 10^4 – 10^6 less than reported lowest adverse effects levels in appropriate in vivo systems. Analogous assumptions can be used to establish a maximum modeled exposure of some better-studied and more toxic mycotoxins (not reported in offices or residences) to serve as a point of comparison. If molds produced the following mycotoxins in offices or residential environments, modeled exposures for aflatoxin B_1, ochratoxin A, and T-2 toxin would be 1.2 x 10^{-6} mg/kg, 1.0 x 10^{-4} mg/kg, and 9.1 x 10^{-5} mg/kg, respectively. For aflatoxin B_1 and ochratoxin A, the modeled exposures are at least two orders of magnitude below those considered safe by the USFDA for the most sensitive human population (children) by ingestion and by WHO for the most sensitive species by ingestion, respectively. For T-2 toxin, the modeled exposure for 24 hours is approximately 10^{-4} less than the estimated human acute oral LD_{50}. Ingestion data were used because quantitative inhalation data are not currently available. It appears that even when using extremely conservative estimates, it is nearly impossible to inhale sufficient mycotoxin in office and residential environments to produce meaningful toxic effects.

1309 EVALUATION OF THE SUBCHRONIC TOXICITY AND ONE GENERATION REPRODUCTIVE EFFECTS OF A FLUOROALKYLETHYL URETHANE POLYMER.

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A fluoroalkylethyl urethane polymer, used as a carpet protectant, was evaluated for its oral subchronic toxicity and reproductive effects when administered to male and female rats by gavage at dosages of 0, 50, 250, or 1000 mg/kg/day. No compound-related mortality occurred in the study. No adverse clinical signs of toxicity or neurobehavior changes were observed in male or female rats in any dose group. Hepatocellular hypertrophy was observed after 90 days of dosing in male rats at all

dose levels. Nasal olfactory epithelium degeneration/necrosis was observed in male and female rats at all dose levels and inflammation of the olfactory epithelium in male rats dosed with 250 and 1000 mg/kg/day. Thyroid hypertrophy was seen in male and female rats in the 250 and 1000 mg/kg/day dose groups. After a 90-day recovery, hepatocellular hypertrophy was still present in male rats in the 250 and 1000 mg/kg/day dose groups. Nasal epithelial lesions were absent and evidence of reversibility of thyroid effects was observed after the recovery period. No test substance-related effects on reproductive parameters were observed. However, mean thyroid organ weight was significantly lower in the 250 and 1000 mg/kg/day dose groups in the F1 generation. There were no histopathological changes in the thyroid of the F1 generation rats. Due to the presence of nasal lesions, a no-observed-adverse-effect-level (NOEL) could not be determined for the subchronic toxicity evaluation. For reproductive assessments a NOEL of 50 mg/kg/day was determined based on decreased thyroid weights observed in both male and female F1 adults. It could not be determined whether the nasal lesions observed are due to systemic toxicity or local toxicity as a result of gavage dosing. Based on the other systemic effects observed at 250 mg/kg/day and above, the potential risk of human health effects from exposure to this product is very low.

1310 EVALUATION OF THE SUBCHRONIC TOXICITY AND ONE GENERATION REPRODUCTIVE EFFECTS OF A FLUOROALKYLETHANOL MIXTURE.

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The objective of this study was to evaluate the subchronic and reproductive toxicity of a fluoroalkylethanol mixture which is employed as an intermediate in the production of other fluoroorganic compounds utilized as protectants and surfactants. Test material was administered by gavage at dosages of 0, 25, 100, or 250 mg/kg/day. No test substance-related mortality or neurotoxicity occurred in the study. Body weights and/or nutritional parameters were significantly reduced at 100 and 250 mg/kg/day but similar to control after recovery. Broken and absent teeth were observed at 250 mg/kg/day. Microscopic tooth lesions (ameloblast degeneration/disorganization) occurred at 100 and 250 mg/kg/day and persisted after recovery. Plasma and urine fluoride levels were elevated in a dose-dependent manner. Decreased red cell mass parameters occurred at 250 mg/kg/day and persisted after 36 days of recovery. The 100 and 250 mg/kg/day groups had increased ($p < 0.05$) liver weights which persisted in the high dose after recovery and correlated with microscopic hepatocellular hypertrophy in males and females (250 mg/kg/day subchronic animals only). Hepatic β-oxidation was increased in a dose-dependent manner and persisted after recovery at 250 mg/kg/day. Increased kidney weights occurred at 25 (females only), 100 and 250 mg/kg/day which persisted in the high dose after recovery and correlated with microscopic tubular hypertrophy (males only). Thyroid follicular hypertrophy was present at 100 and 250 mg/kg/day but was not present after recovery. The test material contains 2% residual iodides. Litter size at birth and pup weights during lactation were reduced at ≥ 100 mg/kg/day. No other reproductive parameter was affected. There were no adverse effects observed for either subchronic or reproductive toxicity in animals dosed with 25 mg/kg/day. A wide margin of safety exists between potential human exposure levels and the doses which result in adverse effects.

1311 DIAMMONIUM PHOSPHATE EMPLOYED AS A CIGARETTE INGREDIENT: 13-WEEK CIGARETTE SMOKE INHALATION STUDY IN THE RAT.

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Diammonium phosphate, $(NH_4)_2HPO_4$ [DAP], is employed as an ingredient in some cigarette tobacco blend components to enhance their organoleptic qualities through reaction with sugars to produce desirable flavoring compounds. The biological responses of male and female Fischer-344 rats exposed nose-only to smoke from filtered control cigarettes were compared to those of rats similarly exposed for 13 weeks to the smoke of test cigarettes containing DAP added directly to the tobacco blend or to a blend component in a manner resembling that employed in contemporary cigarette manufacturing. Test cigarettes contained DAP at five target levels of addition ranging from 882 to 10, 882 ppm in the final tobacco blend. Exposures of 350 mg/m^3 mainstream cigarette smoke were conducted for 1 hr/day, 5 days/week for 13 weeks. The cigarettes' smoke particulate and vapor phase nicotine yields were unaffected by any of the DAP additions. All smoke-exposed groups exhibited similar reductions in body weight gain and increases in blood carboxyhemoglobin, serum nicotine and cotinine relative to the sham control. Smoke-associated changes in respiratory rates and volume, clinical chemistry/hematology, organ weights, gross necropsy findings, and respiratory tract histopathology did not differ substantially among all smoke-exposed groups. All smoke-exposed groups exhibited a similarly reduced incidence and severity of respiratory tract histopathologic findings after a 6-week recovery period following the smoke exposure period. It is concluded that the addition of DAP to experimental cigarettes at levels resembling or exceeding those reportedly employed in contemporary cigarette manufacture does not significantly affect a variety of indices of smoke inhalation exposure or effect in this subchronic rat model.

1312 SAFETY ASSESSMENT OF HIGH FRUCTOSE CORN SYRUP (HFCS) AS AN INGREDIENT ADDED TO CIGARETTE TOBACCO.

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Cigarette design has evolved over a century, with many new technologies introduced in the last several decades. Modifications to cigarette designs have historically involved the incorporation of new ingredients, papers, filters, and tobacco processes. The majority of ingredients are used to enhance aroma and flavor. Others are used to facilitate tobacco processing, facilitate cigarette manufacturing, and preserve moisture levels in the finished cigarette. RJRT has developed a tiered testing strategy to evaluate the potential for new ingredients, tobacco processes, and technological developments to increase or reduce the biological activity of cigarette smoke or cigarette smoke condensates (CSC). The core of RJRT's product stewardship program is comparative testing, typically including chemical and biological components. This comparative approach characteristically relies on the comparison of data from a test cigarette, incorporating the change, as compared to data from an appropriate control. As part of the ongoing stewardship efforts, cigarettes produced with high fructose corn syrup (HFCS), as added tobacco casing material, were subjected to an extensive evaluation. Principal components of this evaluation were an analysis of selected mainstream smoke constituent yields, an Ames assay in Salmonella typhimurium strains (TA98 and TA100), a sister chromatid exchange (SCE) assay in Chinese hamster ovary (CHO) cells and a neutral red cytotoxicity assay in CHO cells, a 30-week dermal tumor-promotion evaluation of cigarette smoke condensate in SENCAR mice, and a 90-day inhalation study of cigarette smoke in Sprague-Dawley rats. The results from these studies demonstrate that the addition of HFCS as an alternate tobacco casing material, up to an inclusion level of 3% dry weight in the final blend, did not produce a differential response in smoke chemistry or biological activity when compared to CSC or mainstream cigarette smoke from control cigarettes without added HFCS.

1313 CALAMUS:REASSESSMENT OF CARCINOGENICITY.

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The flavoring agents, Calamus acorus and its constituents, were considered carcinogens because of reported association of ingestion in feeding and gavage studies and intestinal (mesenchymal) cancers in the 1960's. Recent introduction of Calamus in dietary supplements again raises questions of potential hazards of ingesting Calamus. Osborne-Mendel rats were dosed with oil of calamus or *beta* ascarone, an alkylbenzene component of the oil, and not the plant itself. Often neither the species of Calamus was recorded or the level of ascarone, which ranges from 0 to 96% of the oil, depending on the cultivar and season, with the oil constituting up to 10% of the plant. Extant internal FDA reports reveal flaws in experimentation and interpretation. Absence of complete protocols, small numbers of rats used in the experiments, respiratory disease, poor survival, inadequate sampling of tissues for microscopic examination, and inadequate tissue preservation preclude definitive interpretation. Cardiac and hepatic lesions with chronic passive hyperaemia, growth depression, and mortality were reported in 18-week studies and most of the dose-response relationship claimed in 2-year carcinogenicity studies were in rats dosed well above the 18-week LD50. Examination of archived slides revealed pleomorphic, mesenchymal tumors, some similar to fibrous histiocytomas, a spontaneously occurring tumor type in Osborne-Mendel rats. Bias in over reporting tumors in experimental animals may explain why a positive control in one set of experiments was negative in another set. Other studies of carcinogenesis in mice after intraperitoneal injection of *beta* ascarone in 1987 and in 1999 described palpable hepatomas; but no histologic confirmation was made. The only known effect in humans is protracted emesis after ingestion of a whole rhizome. The relevance of these studies to occasional human oral ingestion of *beta* ascarone in the parts per billion range used for flavoring is equivocal. Available evidence is inadequate to establish the carcinogenic potential of Calamus acorus and its constituents.

1314 PEROXIDE DEGRADATION KINETICS DURING USE OF CREST WHITESTRIPS.

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Oral cavity and systemic exposure to peroxide are important measures of tooth whitening safety. This clinical trial evaluated the hydrogen peroxide (HP) degradation kinetics of 6.0% HP Crest Whitestrips (CWS) on the device, tooth and in saliva during use of this polyethylene strip-based tooth bleaching system. This was a single center, cross-over, 12 subject trial where only maxillary teeth were treated. Device, tooth, and saliva peroxide concentrations were determined at four time points: 5, 10, 30, and 60 minutes. The median peroxide concentrations on the strips at 0, 5, 10, 30, and 60 minutes wearing time were 6.1, 3.8, 3.4, 2.6 and 1.4%. The median peroxide concentrations on teeth at 5, 10, 30, and 60 minutes of strip wearing time were 4.0, 3.1, 3.0 and 1.7%. The median peroxide concentrations in saliva were 0.017, 0.004, 0.006 and 0.002% at 5, 10, 30 and 60 minutes. The concentration of peroxide on teeth was not significantly different from device. The HP concentration in saliva was significantly different from both device and teeth ($p<0.0001$ using a single ranked test). A comparison was made to a previous study with 5.3% HP CWS, 10% Opalescence (10% carbamide peroxide tooth whitening tray system, ~3.3% HP) and 20% Opalescence (20% carbamide peroxide, ~6.6% HP). Dose-Response relationships were observed for the device and teeth data with the 5.3% and the 6.0% HP CWS, as well as with the 10% and 20% Opalescence products. Overall, median salivary HP concentrations were less than 0.02% for any product at any time point measured. This clinical research confirms the safety of CWS by demonstrating the minimal systemic exposure and rapid degradation of HP during use.

1315 FROM EPIDEMIOLOGY TO THE GENE: MECHANISMS BY WHICH PARTICULATE MATTER INDUCES CARDIOVASCULAR EFFECTS.

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Much attention is currently focused on understanding the pathophysiological basis for the observed linkage between ambient air PM exposure and acute mortality. Although there are indications that PM may directly or indirectly affect the cardiovascular system, the biological mechanisms for most of these associations are unknown. A significant effort in PM health effects research has turned towards examining the toxicological mechanisms that underlie PM systemic effects. Recent epidemiology, clinical, and toxicology studies suggest that PM can induce changes in the autonomic control of the heart as well as alter blood components involved in the acute phase response, clotting and coagulation. This symposium will draw on recent cutting edge approaches that investigate PM effects of the cardiovascular system and will integrate information from panel, controlled human exposure, animal and *in vitro* studies. The first two presentations will describe how PM induces changes in heart rhythm and blood components involved in inflammation, the acute phase response, clotting, and coagulation. The next two presentations will describe changes in the cardiovascular system of animals exposed to PM, and will contrast these results with changes found in humans. The final presentation will explore *in vivo* and *in vitro* cardiac molecular effects produced by combustion PM

1316 CHANGES IN CARDIAC BIOMARKERS ASSOCIATED WITH AMBIENT PARTICLES.

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Ambient particles have been associated with exacerbation of cardiovascular disease. However, the biological mechanisms leading from the deposition of ambient particles to acute coronary disease are still to be elucidated. A panel of 58 patients with coronary artery disease has been studied in Erfurt, Germany during the winter of 2000/2001. The goal of the study is to quantify the role of fine and ultrafine particles in elicitation of an acute phase response and changes of the autonomous nervous system function. Repeated clinical examinations were conducted including EKG recording, blood pressure measurements and blood sampling. Ambient particles were characterised by a laser spectrometer and a differential mobility analyser proving particle number concentrations in the size range between 10 nm and 2.5 µm. Multivariate regression models using generalized linear models will be used to quantify the associations between physiological markers such as CRP protein or heart rate variability and ambient particle concentrations in different size ranges adjusting for time trend, metrological variables and viral infections. 683 examinations have been conducted over a half year period, which were more than 95% of the scheduled examinations. EKG readings and blood samples are currently being analysed. The contribution of the study to elucidate the biological mechanisms responsible for the observed health effects of ambient particles within a potentially susceptible subgroup of the general population will be discussed.

1317 CARDIOVASCULAR CHANGES IN YOUNG AND ELDERLY HUMANS EXPOSED TO CONCENTRATED AMBIENT AIR PARTICLES (CAPS).

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Epidemiology studies have shown an association between ambient air particles (PM) and mortality related to cardiovascular causes. In addition, recent panel studies have shown associations between PM and decreased heart rate variability in elderly people with cardiovascular disease. Panel studies have also shown associations between PM and changes in blood factors involved in viscosity and coagulation. The purpose of this study was to determine if exposure of young and elderly volunteers to moderate levels of PM under controlled conditions results in decreased heart rate variability and increased blood factors involved in coagulation. Twenty healthy young volunteers (aged 18 - 35) and 20 healthy elderly volunteers (aged 65 - 80) were exposed to concentrated Chapel Hill ambient air particles (CAPS) for two hours. Heart rate variability was determined before, immediately after, and 24 hrs after exposure. Decreases were observed in both time and frequency domain immediately following CAPS exposure in the elderly, but not young, subjects. No changes were apparent in either group 24 hours following exposure to CAPS. Both young and elderly subjects had increased blood factors involved in coagulation 24 hours after CAPS exposure. These data confirm and extend findings reported in panel studies and suggest a possible mechanism by which PM may cause increased cardiovascular mortality. This abstract of a proposed presentation does not necessarily reflect EPA policy.

1318 EFFECTS OF EXPOSURE TO PARTICULATE AIR POLLUTANTS IN ANIMALS WITH CARDIOVASCULAR DISEASE.

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We have examined the effects of particulate air pollutants (PM) in normal rats and rats with cardiovascular disease to determine whether PM can cause adverse effects in cardiac function in the absence of exacerbation of respiratory disease. Normal young rats, old rats (18 months old), and spontaneously hypertensive rats (SHR) were implanted with either EKG transmitters or blood pressure transmitters. A single 4-6 hr exposure to concentrated ambient PM (CAP) caused a significant change in heart rate in young, old and SHR rats that was not associated with a change in body temperature. No significant change in heart rate was seen in rats exposed to 25 ppm Co., 1 ppm NO2 (SHR rats) or 1 ppm SO2 (young and old rats). Exposure of old and young rats to ultrafine carbon (generated by pyrolysis of acetylene) caused changes in heart rate that were similar to that seen with exposure to CAP. The time course and duration of heart rate change was independent of particulate exposure concentration or particle size (CAP or ultrafine carbon). In young rats, heart rate changes occurred within about 6 hours after exposure and lasted for about 24 hrs but in old rats the onset of heart rate changes lagged about 24 hrs after exposure and was shorter in duration. The old rats were found to have significantly more spontaneous arrhythmias than young rats. Exposure to CAP (but not ultrafine carbon or SO2) significantly increased the frequency of sinus arrhythmias in the old rats. These results suggest that adverse cardiac effects can occur following exposure to PM in rats with underlying cardiac disease without co-existing respiratory disease. Supported in part by EPA R827351

1319 CARDIOPULMONARY CHANGES IN PIGS EXPOSED TO RESIDUAL OIL FLY ASH (ROFA).

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Changes in cardiopulmonary function may contribute to mortality associated with ambient particulate matter (PM). In this study young pigs weighing 30 kg were mechanically ventilated and exposed to 2 mg/kg body weight nebulized ROFA suspended in saline, or to saline alone. Supplemental oxygen was used to maintain O_2 saturation >90%. Changes in heart rate; aortic, central venous, and pulmonary artery pressure; cardiac activation and recovery intervals (ARIs) - an index of cardiac

repolarization - were assessed over a 4 hr period. At that time bronchoalveolar lavage was performed on the cardiac lobe and portions of the lung and heart were removed for immunohistochemical analysis. ROFA-exposed pigs had a 8.3 fold increase in BAL PMNs and a 2.4 fold increase in BAL protein compared with pigs exposed to saline. There was a 4.8 mm Hg increase in pulmonary artery systolic pressure following ROFA exposure compared with a 2.3 mm Hg decrease after saline exposure. In ROFA-exposed pigs, heart rate increased by 19 bpm and ARIs decreased, compared to saline-exposed animals. In this pig model, ROFA increases lung injury and inflammation, pulmonary hypertension, and affects cardiac impulse formation and repolarization. This abstract does not necessarily reflect EPA policy.

1320 BIOAVAILABLE CONSTITUENTS MEDIATE CARDIAC MOLECULAR EFFECTS OF PULMONARY DEPOSITED FUEL OIL COMBUSTION PARTICLES.

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Environmental health effects studies have shown the ability of particulate air pollution to affect cardiac autonomic control. The mechanism(s) by which particulate air pollution alters cardiac physiology are unknown. It is also not known what cardiac molecular effects, if any, may relate to specific combustion particles present within the fine fraction of particulate air pollution. This study examines the ability of fuel oil combustion particles, residual oil fly ash (ROFA), deposited in the lung to induce cardiac molecular effects and determines the mechanism(s) by which these effects arise. Gene expression profiling detected an increase in mRNA levels for several cytokines and matrix metalloproteinases within hearts recovered from ROFA-instilled rats at 1 - 6h and 24h post-exposure. Protein profiling detected an increase in the total phosphotyrosine protein content and the activation of several protein kinases in hearts recovered from ROFA exposed rats as early as 0.25 - 1h post-exposure, indicating a rapid alteration in cardiac intracellular signaling homeostasis. Pulmonary exposure to ROFA did not increase plasma levels of IL-1beta, TNFalpha, IL-10 or endothelin-1. Elevated plasma vanadium (V) content was observed in ROFA-exposed rats as early as 15 min post-exposure which remained elevated up to 6h post-exposure before decreasing towards control saline levels by 24h post-exposure. Exposure of cardiac fibroblast/myocyte co-cultures to a particle free ROFA leachate produced similar molecular responses as observed *in vivo*. These results demonstrated the ability of ROFA to produce cardiac molecular effects that were temporally correlated with plasma V content. These effects occurred rapidly and prior to the development of pulmonary inflammation, and could be replicated *in vitro*. These findings suggest that bioavailable metal constituents are responsible for the cardiac molecular effects following *in vivo* or *in vitro* exposure to fuel oil combustion particles.

1321 TRANSCRIPTIONAL INVOLVEMENT IN NEUROTOXICITY.

N. H. Zawia. *Biomedical Sciences, University of Rhode Island, Kingston, RI.*

It has become more apparent that exposure to various chemicals and environmental hazards elicits changes in the expression of a variety of genes. The study of gene expression and transcriptional regulation is an important aspect of understanding the mechanisms associated with neurotoxicity. The availability of whole genome sequences and the development of new tools to identify and monitor transcriptional activity have accelerated the rate of discovery. This has led to the identification of new genes and the ability to monitor the activity of multiple genes that act in concert in response to a stimulus. This symposium will deal with recent advances related to the elucidation of genes expression associated with the neurotoxic response as well as deciphering of signal transduction/transcription coupling that is altered following exposure to neurotoxic agents.

1322 THE SP FAMILY OF TRANSCRIPTION FACTORS MEDIATE METAL-INDUCED CHANGES OF GENE EXPRESSION.

N. H. Zawia. *Biomedical Sciences, URI, Kingston, RI.*

Sp1 is a transcription factor which contains a zinc finger motif and whose activity is modulated both *in vivo* and *in vitro* by heavy metals such as Pb, Hg, and Cd. Some of the genes under Sp1 control include ornithine decarboxylase, myelin basic protein, proteolipid protein, NMDAR1 subunit, and metallothionein. It has now become apparent that Sp1 belongs to a family of proteins whose members include Sp2, Sp3, Sp4. All the Sp factors recognize the same DNA element. While the role of Sp2 is unknown, Sp3 is suspected of being a negative modulator of transcription and Sp4 exhibits tissue specificity for the brain. The interactions of Sp1 with cell cycle regulatory proteins, including p53, retinoblastoma protein, and cyclins is consistent with its developmental role. Furthermore, Sp1 has been shown to play a critical role during the differentiation of oligodendrocytes in the human brain. This presentation will discuss metal-induced changes in the activity and structure of this transcription factor family. Specific focus will be placed on the different roles of the various members of the Sp family in the regulation of gene expression.

1323 GENE EXPRESSION AND CELL-SIGNALING EVENTS ASSOCIATED WITH TOXICANT-INDUCED GLIOSIS.

J. P. O'Callaghan[1], K. Sriram[1], M. I. Luster[1] and D. M. Felschow[2]. [1]*Molecular Neurotoxicology, CDC-NIOSH, Mogantown, WV and* [2]*Ciphergen Biosystems, Fremont, CA.*

Astrogliosis represents a sensitive and early response of the nervous system to all types of neurotoxic injuries. The generality of the glial reaction, despite the target selectivity of specific neurotoxic insults, implies that there are common "signals" underlying this cellular response. The discovery and characterization of such signals would, therefore, broaden our understanding of molecular mechanisms underlying diverse neurotoxic responses and lead to early "predictors" of neurotoxic outcomes. We present evidence for candidate genes and signaling pathways underlying the glial response to neuronal damage resulting from exposure to the dopaminergic neurotoxicant, MPTP. In our model, a single 12.5 mg/kg dose of MPTP to the C57 Bl6/J mouse results in a 50% decline in dopamine and TH protein. The accompanying astrogliosis, as assessed by immunoassay of GFAP, begins at 12 hrs and peaks at 48 hrs post dosing. Striatal homogenates prepared from mice sacrificed by focused microwave irradiation to preserve steady-state phosphorylation revealed activation of the JAK-STAT pathway in the earliest phase of MPTP-induced gliosis. This effect was not observed in non-target regions and was completely reversed by neuroprotection with nomifensine. These results were indicative of effects specific to the neurotoxic condition and implicated potential upstream effectors in the JAK/MAP kinase modules, such as cytokines and trophic factors acting through the gp130/Ras pathways. Gene array analysis revealed enhanced expression of TNF-α mRNA and the Ciphergen Protein Chips®platform of SELDI-TOF/MS analysis revealed a striatal-specific induction of 18 kD proteins consistent with induction of TNF-α. TNF-α receptor deficient mice showed complete neuroprotection against the neurotoxic effects of MPTP. Together these data show that a gene- protein- and phospho-specific antibody "arrays" can reveal novel mechanisms potentially underlying the glial response to neurotoxic insult.

1324 GENE EXPRESSION PATTERNS IN LEAD NEUROTOXICITY.

T. R. Guilarte. *Environmental Health Sciences, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.*

Human studies and animal models have clearly demonstrated the impact of developmental lead (Pb) exposure on behavior and cognitive function. Experimental animals exposed to environmentally relevant levels of Pb express deficits in tests of spatial learning and in synaptic plasticity such as long-term potentiation (LTP). The hippocampus is a brain structure essential for both of these processes and its known to be affected by Pb exposure. We have been interested in understanding the molecular basis associated with these deficits and have identified changes in the expression of specific subunits of the NMDA receptor in the hippocampus of Pb exposed rats. This presentation will discuss Pb-induced changes in the expression of selective genes associated with NMDA receptor-dependent signal transduction and calcium homeostasis. [Supported by NIEHS grant ES06189]

1325 INDUCTION OF TRANSCRIPTION FACTORS IN NEURONS THAT SURVIVE NEUROTOXICITY.

K. R. Pennypacker. *Pharmacology, USF, Tampa, FL.*

We report that levels of Fos-related antigen-2 (FRA-2) are elevated long-term in several models of chemical-induced brain injury as well as after ischemia suggesting that this protein is involved in enhancing the transcription of genes related to the process of regeneration and repair. Trimethyltin, which causes degeneration of neurons primarily in the hippocampus and other limbic regions, results in a 5-fold induction of FRA-2 immunoreactivity in neurons in the pyramidal and dentate layers of the hippocampus starting at seven days post treatment and persisting for 60 days. Ischemic insult caused by middle cerebral artery occlusion results in an increase in FRA-2 expression the hippocampus ipsilateral to the ischemic lesion at 6 hours

after reperfusion. By one day, the increased expression is found in the neuronal layers of both hippocampi and its elevated expression lasts for at least 21 days. Methamphetamine and methylenedioxymethamphetamine, agents which cause degeneration of dopaminergic nerve terminals in the striatum of the mouse, cause an increase in FRA-2 immunoreactivity which begins at three days post-treatment and returns to basal levels by day 5 and 15, respectively. Treatment with 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine caused elevated levels of FRA-2 in the mouse striatum at three days post-treatment. This abbreviated timecourse of FRA-2 induction in terminal degeneration is consistent with less severe insult (terminal damage) relative to trimethyltin and ischemia (cell death) but induction occurs during the period of regeneration and repair in both models. Thus, FRA-2 is induced in models of both cell death and terminal degeneration suggesting this transcription factor may serve as a universal signal transduction molecule involved in the regulation of genes related to regeneration and repair in the central nervous system. Work supported by AHA Nat. 9930072N and NIH NS39141-01A2.

1326 NUCLEAR FACTOR KAPPAB ACTIVATION PROMOTES SURVIVAL OF MOUSE HIPPOCAMPAL CELLS AFTER ACUTE TRIMETHYLTIN EXPOSURE.

C. A. Kassed and K. R. Pennypacker. *Pharmacology and Therapeutics, University of South Florida, Tampa, FL.*

Members of the nuclear factor-kappaB (NF-$_{\kappa}$B) family of transcription factors play essential roles in diverse physiological processes including cell growth, differentiation, and development. A variety of external stimuli including pathogens, stress, and pharmacological agents can activate NF-$_{\kappa}$B. However, the role of this transcription factor as a neurodegenerative or survival-promoting response to brain injury remains an area of dissension. To address this controversy, we have established transgenic mouse models of neurodegeneration using trimethyltin, the well-characterized neurotoxicant with exquisite limbic selectively. Neurodegeneration is exacerbated in the hippocampi of mice lacking the p50 subunit of NF-$_{\kappa}$B. Activation of this transcription factor in response to an acute, systemic injection of TMT is shown in mice carrying a $_{\kappa}$B-dependent *lacZ* ($_{\beta}$-galactosidase) reporter gene. Granule cells of the murine dentate gyrus subregion are particularly vulnerable to TMT toxicity, but neurons expressing activated NF-$_{\kappa}$B, identified by $_{\beta}$-galactosidase reporter activity, survived the injury seven days after treatment. Additionally, Fluoro-Jade, the histochemical marker of degenerating neurons, does not label cells immunoreactive for $_{\beta}$-galactosidase. These results further support a role for NF-$_{\kappa}$B transcription factor in neuronal survival. This work was supported by grants from American Heart Association National 9930072N and National Institutes of Health NS39141-01A2

1327 APPLICATION OF GENOMICS FOR MECHANISM BASED RISK ASSESSMENT.

R. D. Tyler and D. E. Robinson. *ILSI Health & Environmental Sciences Institute, Washington, DC.*

Recent advances in genomic and proteomic research, coupled with the availability of novel tools and methods with which to analyze the products of altered gene expression, have provided new insights into mechanisms of toxicity evoked by xenobiotics. While these advances promise to revolutionize our ability to characterize hazard, one of the short-term needs is to establish a body of publicly available knowledge to serve as a foundation for applying the data generated by these new methods to risk assessment. The ILSI Health and Environmental Sciences Institute is providing a scientific forum to facilitate further development and advances in genomics and proteomics methodologies toward the goal of increasing the utility of gene expression data for mechanism-based risk assessment. A collaborative research effort has been undertaken to help establish appropriate experimental methodologies for measuring alterations in gene expression and to relate genomic and proteomic expression data to toxic endpoints characterized by established methods under standardized conditions. Through this collaboration, data are being generated in government, industry and research laboratories on a series of prototype compounds across a broad range of available microarray platforms. This workshop will present the outcome of the initial phase of this project which has focused on nephrotoxicity, hepatotoxicity, and genotoxicity as important endpoints for the risk assessment of many chemicals. Presenters will discuss the issues associated with drawing correlations between patterns of gene expression with key biological parameters for these endpoints, insights gained into the mechanism of action of the compounds studied, the influence of specific experimental conditions on data variability and reproducibility, and other issues associated with interpretation and future application of data from these types of studies for risk assessment.

1328 FUTURE DIRECTIONS FOR SCIENTIFIC VALIDATION AND APPLICATION OF GENOMICS APPROACHES IN RISK ASSESSMENT.

W. D. Pennie. *Syngenta CTL, Macclesfield, United Kingdom.* Sponsor: R. Tyler.

The ILSI Health and Environmental Sciences Institute is providing a scientific forum to facilitate further development and advances in genomics and proteomics methodologies toward the goal of increasing the utility of gene expression data for mechanism-based hazard identification and possibly risk assessment. One of the short-term goals of this endeavour is to establish a body of publicly available knowledge to serve as a foundation for assisting in the generation, interpretation and application of data generated by these new methods. To this end, a collaborative research effort has been undertaken to help establish appropriate methodologies for measuring alterations in gene expression by developing and applying standard protocols across multiple technical platforms. The initial phase of this project has focused on nephrotoxicity, hepatotoxicity, and genotoxicity as important endpoints for the risk assessment of many chemicals. The successful completion of these initial studies has raised important technical issues relating to microarray applications including experimental design, sources of variability, gene annotation, analysis methods and the sharing and comparison of results from different technical platforms. To facilitate the sharing of multi-platform results, the ILSI subcommittee has developed short-term microarray data exchange and storage formats, which allow simplistic comparisons between multiple datasets. It is clear that the most likely value from these approaches will be obtained when they are applied in concert with more "traditional" measurements of toxicant action such as well-characterized biochemical or histopathological markers or endpoints. In addition the successful interpretation of changes in global patterns of gene expression will be greatly facilitated by the availability of an appropriately curated and carefully annotated public domain database of gene expression data with simultaneous recording of appropriate phenotypic markers related to the effects of chemical exposure. Ongoing effort and challenges in these areas will be presented.

1329 GENOMIC AND PROTEOMIC ANALYSIS OF ACUTE AND SUBCHRONIC NEPHROTOXICITY.

T. A. Bertram. *Drug Safety, Pfizer, Groton, CT.*

Pharmaceutical agents account for about 40% of clinically relevant acute renal failures/nephrotoxicity accounts for up to 6% of regulatory actions related to target organ toxicity. Nephrotoxicity is associated with 4 basic mechanisms. These mechanism are active on the Glomerulus; Tubules/collecting ducts; &/or Interstitium. The Nephrotoxicity group of ILSI's Genomics Subcommittee tested dose-/time-response relationships of 3 agents in SD rats: Gentimycin; Cisplatin; & Puromycin. 4 genomic platforms were used to evaluate mRNA expression. Common sources of variation in genomic analysis included: inter-laboratory in-life studies, inter-laboratory m-RNA array analysis, inter-animal & intra-array effects. Genes were found to be regulated in treated animals at all times. Microarray analysis demonstrated temporal/dose-response for genes functioning in cellular injury, molecular repair, intercellular signaling, cellular proliferation, apoptosis, inflammation, fibrosis, electrolyte transport & acute phase response. Classical gene markers for glomerular injury (vimentin), proximal convuluted tubular damage (gamma glutamyl transpeptidase), collecting duct toxicity (Na-H exchanger), & interstitial damage (KIM-1) were identified using microarray technology. Patterns of gene responses for known toxic mechanisms & functional categories were consistent with what is known about the mechanism of nephrotoxicity. Potential applications from genomic & proteomic studies of nephrotoxicity include early candidate selection & prioritization, animal model development, elucidation of mechanisms for tissue injury & response, and interspecies extrapolations.

1330 GENE EXPRESSION MONITORING USING MICROARRAYS AS AN APPROACH FOR ASSESSING HEPATIC TOXICITY.

R. G. Ulrich. *Abbott Laboratories, Abbott Park, IL.* Sponsor: R. Tyler.

Hepatic toxicity is the most common adverse effect leading to label warnings, use restrictions and market withdrawals for pharmaceuticals. Traditional experimental approaches can reveal dose-dependent toxicity in animal models but rarely indicate toxic mechanism and are equivocal predictors for human responses. Microarrays and related tools have shown promise for the rapid identification of toxic mechanism and may have utility in more precisely predicting human responses. To be useful for risk assessment these tools must be challenged for reliability and inter-laboratory reproducibility of results. Towards this, the hepatotoxicity working group of the ILSI Genomics Subcommittee is evaluating responses in rats to two hepatotoxins using a standard experimental protocol. Clofibrate (25 and 250 mg/kg/d) and methapyrilene (10 and 100 mg/kg/d) were administered by oral gavage for 1, 3 and

7 days. Studies for both compounds were conducted in two different laboratories, and traditional toxicity parameters were consistent with reported effects. RNA samples from liver were distributed for examination in 16 different laboratories using six different platforms and at least three different data analytical approaches. Gene expression results for both compounds show sufficient agreement between laboratories and across platforms to show promise for this new approach to toxicity evaluation. In most cases expression of known marker genes for these compounds was observed. Discrepancies for specific genes are largely due to differences in platforms (gene representation, annotation and specific sequences) and approach to data analysis rather than biological or inter-laboratory variability. This consortium effort provides an unprecedented opportunity to evaluate and compare biological, genomic and toxicological parameters across different laboratories and different analytical platforms. Though improvements are needed in annotation and in data analysis tools, it is clear that monitoring gene expression changes has value for identifying and assessing hepatic toxicity.

1331 ASSESSMENT OF THE UTILITY OF DNA MICROARRAYS AS A PRACTICAL TOOL FOR CHARACTERIZING GENOTOXICITY.

R. K. Newton. *Investigative Toxicology, Eli Lilly, Greenfield, IN.* Sponsor: D. Robinson.

Genetic damage elicits a variety of stress-related signals that alter expression of genes associated with numerous biological pathways. It has been proposed that patterns of gene expression changes may be characteristic of specific classes of genotoxic compounds and can be used to classify agents with different mechanisms of action. DNA microarray platforms provide a promising technology to assess these changes. To test the hypothesis that gene expression patterns can be correlated with mechanisms of action of genotoxicants, the ILSI Genomics Subcommittee has investigated the gene expression profiles of several direct-acting genotoxins in two different cell lines (mouse lymphoma and human TK6 cells). This multi-laboratory collaboration has afforded the opportunity to assess several factors of inter-laboratory variability associated with various aspects of the technology. Two time points were examined: 1) cells were treated for 4 hr and immediately harvested for RNA isolation, or 2) cells were treated for 4 hr and allowed to recover for 20 hr (24 hr time point) before being harvested. At least two concentration levels were chosen with one inducing 70-80% cytotoxicity with a robust induction of genotoxicity (determined by either a TK gene mutation assay or a micronucleus assay) or one inducing limited cytotoxicity with a 2-4 fold genotoxic response. Overall, the data indicates that modest changes in gene expression (<3-fold) were common in the *in vitro* studies, and levels of test compound that produce low cytotoxicity appear to induce relatively few gene changes. Interpretation of the data is influenced by the filter criteria used to define a response. At present, it is uncertain that these modest changes represent key biological responses to damage in these cell lines or if other mechanisms apply. To fully utilize information from these studies, it will be critical to analyze data in a manner that identifies critical genes in biological pathways involved in a genotoxic response and to distinguish small, but real changes from background noise.

1332 USING MICROARRAY TECHNOLOGY IN SAFETY EVALUATION: WHERE DO WE GO FROM HERE?

L. L. Stevens, D. E. Watson and T. P. Ryan. *Toxicology and Drug Disposition, Lilly Research Laboratories, Indianapolis, IN.*

Expression profiling by microarray analysis significantly contributes to many areas of Toxicology including biomarker development and mechanistic studies. However, the promise represented by microarray analysis must be balanced against the significant challenges that exist at all levels of the technology. For example, the quality of the data must be consistent from chip to chip and Lab. to Lab.. In addition, for public data to be widely disseminated, hardware and software systems must manage and deliver complex data sets from many sources and platforms to multiple users *via* internet links. Additional complexity occurs in interpreting results. One must know the function of a gene to interpret the biological significance of a change in expression. Thus, functional annotation of genes is a significant hurdle for the field since correct assignment increases the likelihood that changes in gene expression will yield useful biological information. Unfortunately, much of the transcriptome is not annotated nor has function been assigned to many novel genes. In addition, nomenclature often does not reflect function. These problems represent significant challenges if we are to develop databases into which many users can deposit data and from which useful biological information can be extracted. Nonetheless, reference sets of expression profiles developed around defined SARs do offer insights into mechanism, suggesting that databases of gene expression information have applications in risk assessment. The ILSI effort on microarray technology highlights the promise and problems inherent to microarray technology. Continued effort will contribute to international databases and provide a benchmark for standardizing microarray technology within the industry.

1333 APPROACHES TO THE ASSESSMENT OF FOOD ALLERGENICITY.

G. S. Ladics[1] and W. Dong[2]. [1]*DuPont Co., Haskell Laboratory, Newark, DE and* [2]*USFDA, Washington, DC.*

Food allergy is an important health issue with the prevalence of IgE antibody-mediated food allergies among adults being approximately 2% and nearly 5% in children. There is a need to assess the safety of foods deriving from genetically modified (GM) crops, including the allergenic potential of novel gene products. In January 2001, a Joint FAO/WHO Consultation developed a revised decision tree for the evaluation of the allergenic potential of GM foods. In addition to indirect measurements of allergenic activity (amino acid sequence homology and structural similarity to known human allergens, together with resistance to pepsin digestion), the new hierarchical approach now recommends confirmation using serological screening (i.e., using targeted evaluation of the reactivity of novel proteins with IgE antibody from sensitized subjects) and relevant immunological assays. Presently, there are no *in vitro* or animal models that have been validated for the identification or characterization of potential protein allergens. This Workshop will gather researchers working on various strategies for assessing protein allergenicity to describe the current state of knowledge and progress that has been made in the development and evaluation of appropriate testing strategies and to identify critical issues that must now be addressed. *in vitro* models of digestibility, bioinformatics, and risk assessment in the context of food allergy prevention and management clinically will be discussed. Both rodent and non-rodent models for the measurement of allergenic potential of GM foods will also be reviewed.

1334 ALLERGY ASSESSMENT OF GENETICALLY MODIFIED (GM) FOODS: WHAT ARE THE ISSUES?

M. P. Holsapple. *TERC, Dow Chemical Company, Midland, MI.*

Prior to marketing, GM products are required to be evaluated for their safety, including the potential for allergenicity. The process by which allergy assessment is conducted has evolved under the guidance of a variety of expert scientific bodies, and the key steps in the current decision tree include the following: Is the source of the gene allergenic? (addresses the history of exposure and safety of the source of the gene); Is the amino acid sequence similar to any known allergen? (addresses the potential for broad homology of the protein to known allergens; search for 8 or more contiguous amino acids); Is the protein stable to pepsin digestion? (addresses the potential for absorption through the stomach). To date, approximately 70 GM products have been assessed for safety and approved for food use in at least one country by regulatory authorities. While the current decision tree has been successfully applied, there are scientific issues requiring additional clarification including the following: the need to standardize the amino acid sequence analysis; the uncertainty about whether IgE epitopes are missed by the current search criteria; the need to standardize the pepsin digestion assay; the need for scientific consensus on what to do with stable proteins; the need for scientific consensus regarding the usefulness of broad serum screens; the need for more research to validate predictive animal models. The FAO/WHO 2001 report addressed many of these issues and made recommendations that could provide additional reassurance of allergenicity assessment. However, some of these recommendations cannot presently be integrated because of the unavailability of validated methods. Any change in the current methodology should include a development plan with clearly defined goals and criteria. The FAO/WHO 2001 decision tree does not allow for a GM food to be devoid of allergenic risk - the best scenario is a low probablility of allergenicity, which introduces a degree of uncertainty. Any safety assessment approach should enable its practioners to make clear, consistent decisions based on sound scientific evaluations.

1335 FACTORS RELEVANT TO THE ALLERGY ASSESSMENT OF FOODS DERIVED FROM BIOTECH CROPS.

J. D. Astwood, R. E. Goodman and E. A. Rice. *Product Safety Center, Monsanto Company, St. Louis, MO.* Sponsor: W. Dong.

Protection of food allergic subjects from unwelcome exposures to the proteins which cause their clinical symptoms represents a major public health priority for plant biotechnology. Successful methodologies for identification of established al-

lergens have been utilized since the introduction of the first biotech crops in the early 1990's. Recent progress in the understanding of both the natural history of food allergy and the characteristics which define food and other allergens has lead to new insights into the potential predictive value of *in vitro* tools with a view towards preventing the development of new food allergies. The most advanced of these tools include 1) the adaptation of bioinformatics techniques; and 2) the refinement of two factors which influence protein exposures *via* foods: stability to digestion and protein levels. The positive and negative predictive values of these factors alone and in combination will be discussed. In addition, emerging trends in the characterization of food and other allergens, especially allergens responsible for Oral Allergy Syndrome (OAS), will be reviewed with a view to enhancing the risk assessment approach for foods derived from biotech crops.

1336 ANIMAL MODELS FOR THE IDENTIFICATION OF PROTEIN ALLERGENIC POTENTIAL: THE BALB/C MOUSE.

I. Kimber and R. J. Dearman. *Research, Syngenta Central Toxicology Laboratory, Cheshire, United Kingdom.*

Food allergy is an important and common health issue, and there is consequently a need to identify and characterize the sensitizing potential of novel food proteins. One strategy we have adopted is to measure allergenic activity as a function of the ability of proteins to provoke antibody (IgG and IgE) responses in BALB/c mice; a strain that is known to favour adaptive immune responses of the quality required for allergic sensitization. Emphasis has focused to date on an examination of humoral immune responses induced following systemic exposure of mice (by intraperitonal [ip] administration) to the test material. Under these conditions it has been found that proteins known to be associated with food allergy in humans (including for instance peanut proteins and ovalbumin) elicit both specific IgG and IgE antibody responses, measured using enzyme-linked immunosorbent and passive cutaneous anaphylaxis assays, respectively. In contrast, other proteins derived from foods believed to exhibit little or no allergenic potential (such as for instance an extract of potato proteins), although immunogenic in BALB/c mice (measured as a function of IgG antibody production) either failed to induce IgE responses at all, or were associated with only low grade IgE production. In comparative analyses, exposure of BALB/c mice to proteins by gavage was found to be less sensitive and discriminatory. Experience to date indicates that the inherent sensitizing potential of novel food proteins can be evaluated (identified and ranked) on the basis of antibody responses stimulated by parenteral exposure of BALB/c strain mice.

1337 DETERMINATION OF PROTEIN ALLERGENICITY: STUDIES IN A BROWN NORWAY RAT FOOD ALLERGY MODEL.

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There is a need to identify and characterize the potential allergenic activity of novel proteins introduced into genetically engineered crops. Although structural and physico-chemical approaches have been described, including comparison of sequence and structure to known allergens, determination of the stability to digestion with pepsin, stability with heat, and the presence of glycans or disulfide bonds; none of these directly measure the ability of proteins to cause allergic sensitization. For that reason there has been a growing interest in the development of suitable predictive animal models. Although several animal models are under development, none of these models have been widely tested with a variety of allergenic and non-allergenic proteins. In our rat food allergy model, we expose young Brown Norway (BN) rats (high IgE responder strain) to food proteins by daily gavage dosing for 42 days without the use of adjuvants. The animals develop specific IgE responses against known food allergens. After an oral challenge in previously sensitized animals, increased gut permeability, drop in blood pressure, drop in breathing frequency, and increased release of Rat Mast Cell Protease II *in vivo* was observed. In recent studies with the BN rat food allergy model the animals were sensitized to common strong and a non-allergenic protein to study the potential allergenicity of food proteins. Sensitizations were attempted orally both with and without the use of the oral adjuvant Cholera Toxin (CT) and parenterally with the use of alum as an adjuvant. The results indicate that at day 42 the immune responses in the BN rat can distinguish between strong and non-allergenic proteins. Although none of the proteins induced 100% of the animals to respond after oral exposure there was a difference in response observed between the proteins as measured by either antigen and isotype specific ELISA and by PCA.

1338 NON-RODENT ANIMAL MODELS FOR ASSESSING PROTEIN ALLERGENICITY.

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In veterinary medicine, skin and gastrointestinal reactions to foods are commonly diagnosed in domestic animals such as pigs, calves, and dogs. An inbred Spaniel/Basenji colony with a genetic predisposition, significant history and cutaneous signs consistent with food-induced allergic dermatitis has been selected to study food-induced IgE-mediated allergy. Newborn pups, subcutaneously sensitized with allergens in alum and vaccinated with attenuated distemper-hepatitis vaccine respond with allergen-specific IgE, skin test reactivity, and histologic features that mimic human food allergy. A significant contribution of this model is that it mimics a proposed mechanism in children representing viral infections and a protein "bystander" antigen response. Young pigs experience allergic reactions to legume proteins and represent a naturally occurring food allergy model for studying food sensitization and gut-immune mediated disorders resembling human food allergic diseases. The pig mimics the human digestive and gut immunologic functions, including nutritional requirements, a transient porosity of the immature gut to dietary proteins, the distribution and maturation of intestinal enzymes, and the enteric absorption of antibodies. Neonatal piglets sensitized by either an intraperitoneal or intragastric administration of peanut allergens in the presence of adjuvant followed by intragastric challenge with peanut meal provokes emesis, skin rashes, lethargy and respiratory distress. These two non-rodent animal models demonstrate skin test reactivity when challenged with the relevant allergen, show pronounced mucosal swelling and persistent erythema following endoscopic analysis of food challenges that is similar to that of human subjects undergoing diagnosis for food allergy. Continued efforts in these non-rodent animal models will provide valuable insight into the mechanisms of IgE-mediated allergic disease and be able to assist in the prediction of the relative allergenicity of novel proteins.

1339 EXPRESSION PATTERNS OF ORTHOLOGOUS SEQUENCES IN MOUSE AND HUMAN.

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The comparison of two genomes provides insight into the function and organization of the shared genes. A genome wide comparison of available human (95%) and mouse (15%) sequence was performed to identify putative orthologous genes. Approximately, 95, 000 conserved elements were identified that were greater than 100 bp and 62% of these regions contained an open reading frame. The mean identity of the conserved elements was 87.2 ± 4.2% which is consistent with previously described orthologous transcripts between both human and mouse. A subset of 2, 000 putative orthologous elements were spotted on tandem microarrays (one human and one mouse) and used to evaluate conservation of expression patterns between organisms. Across a panel of 9 tissues, gene expression between mice and humans was largely conserved with 92% expressed with less than 6-fold difference between organisms and 74% expressed with less than 3-fold different. Of the genes expressed with greater than 10-fold difference, the majority were genes of unknown function (42%), followed by signal transduction (24%), metabolism (12%), and cytoskeletal (10%). Although this study extends the comparison between mouse and man from the genome to the transcriptome, it highlights the potential differences between these two species and provides an approach for evaluating cross-species extrapolation issues in toxicology at the molecular level. Preliminary experiments show similar transcriptional responses to phorbol esters between human HepG2 and mouse Hepa1 cells. Additional experiments are underway to compare human and mouse transcriptional responses to a variety of chemical treatments. We propose that such orthologous arrays will be useful for evaluating animal models for relevance to both human disease and toxicology.

1340 INTERSPECIES COMPARISON OF HEPATOTOXIC STRESS RESPONSE USING MICROARRAYS OF ORTHOLOG CDNA FRAGMENTS.

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The rat is extensively used in toxicological studies, partly, because the degree of evolutionary divergence is low enough to allow a cross-referencing to human and rat responses with respect to toxicological endpoints. Therefore, cross referencing molecular transcriptome data from studies with rat and human probes has many important applications. We have investigated the expression pattern of 1007 orthologues genes that code for detoxification, cell proliferation, tumor development,

heat shock response, signal transduction, apoptosis and cell cycle regulation after treatment of primary human and rat hepatocytes with a set of toxic compounds, e.g. Aroclor 1254. cDNAs were assigned into gene families and a suitable subsequence was selected fulfilling a variety of quality parameters. First the sequence architecture was verified by cross-species comparison. The second step included the masking of repetitive elements as well as vector sequences and the region beyond the first possible polyadenylation signal. After analysis of putative alternative splice events and the removal of ambiguous regions, the remaining sequence was used for similarity searches. Finally, a fragment was generated by RT-PCR using gene specific primers from the part of the sequence that has no apparent risk for cross-hybridization with other closely related genes and is sufficiently well conserved between human and rat to allow amplification of fragments from the same region of a given gene from both species. These cDNA fragments from human and rat were used to produce species specific microarrays and probed with human and rat hepatocytes respectively, which have been treated under the same conditions. An interspecies correlation of orthologe genes with respect to their transcriptional regulation is presented. Our results have various implications concerning the predictive power of toxicology studies using primary rat hepatocytes.

1341 HEPATIC GENE EXPRESSION PROFILES IN MICE TREATED WITH LPS: COMPARISON OF WHOLE LIVER AND LASER CAPTURE MICRODISSECTED HEPATOCYTES.

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We investigated the feasibility of using laser capture microdissected (LCM) material for Affymetrix gene expression profiling (GEP). Approximately 12, 500 cells were dissected from randomly selected areas of sectioned lipopolysaccharide (LPS) or saline-treated mouse livers. Total RNA extracted from these cells was found to be intact and of good quality when analyzed with the Agilent Technologies 2100 Bioanalyzer. A modification of published procedures was used to label 50 ng of RNA from the 12, 500 LCM cells. For comparison to the LCM samples, total RNA was also extracted from intact liver and either labeled directly according to the standard Affymetrix protocol (10 ug) or diluted (50 ng) and labeled with the modified protocol. All samples were hybridized to Affymetrix murine U74A GeneChips and analyzed by a variety of statistical methods. Multidimensional scaling analysis revealed more similarity among samples analyzed using the modified amplification protocol compared to the standard protocol. This is most likely due to the 3' bias that is introduced in the amplified samples from the random hexamers and oligo-dT priming used in the second round of cDNA synthesis. Importantly, however, when control and LPS-treated samples were compared for differential gene expression profiles, the patterns in both the unamplified (i.e. undiluted liver RNA) and amplified (i.e. diluted liver and LCM liver RNA) samples were remarkably similar. In all samples genes encoding several acute-phase and other LPS-responsive proteins were up-regulated, and numerous cytochromes P450 were down-regulated. Additional results comparing profiles generated from 500 cells and from hepatocytes isolated from centrilobular and periportal regions control and LPS-treated livers will also be discussed. The conservation of differential gene expression profiles generated from small amounts of RNA demonstrates that this approach should have broad applicability to the investigation of molecular mechanisms of toxicity and disease.

1342 THE USE OF cDNA MICROARRAYS FOR ASSESSING HEPATOTOXICITY - AN INTER LABORATORY ASSESSMENT.

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cDNA microarray technology has the potential to have a significant impact on our ability to identify toxic hazards by the identification of mechanistically-relevant markers of toxicity. First however, an assessment of the reproducibility of the data generated must be undertaken. As part of the Hepatotoxicity Working Group of the ILSI/HESI Genomics Subcommittee, the biological response in rats to the model hepatotoxin clofibrate, was investigated. Animals were treated with high (250mg/kg/day) and low (25mg/kg/day) doses of clofibrate by oral gavage for a period of 1, 3 and 7 days. Following sacrifice, livers were removed for histopathological assessment and total RNA (pooled) prepared for distribution to participating laboratories for analysis. This presentation focuses on gene expression data obtained using Atlas™ Rat Toxicology II arrays (Clontech). Samples were processed on triplicate arrays using ^{33}P labelled cDNA probes. Probed arrays were exposed to X-ray film or phosphor screens and images captured and analysed using AtlasImage™2.0 software. Further statistical analysis of the data was performed by each laboratory. In general, there was a good degree of concordance between the gene expression profiles generated at each site. Expected changes in gene involved in fatty acid metabolism (e.g. acyl CoA oxidase, hydroxyacyl CoA dehydrogenase, 3-ketoacyl CoA thiolase A/B), cell proliferation (e.g. topoisomerase II-α) and metabolism (CYP4A1) were observed. Real Time PCR was used to confirm modulations for selected genes. Although some discrepancies in microarray data between the two laboratories were observed, they appear largely due to different approaches to image and data analysis. In conclusion, toxicogenomics has the potential to provide new and refined approaches to hazard identification and safety evaluation based on the identification of biologically relevant markers of toxicity.

1343 GENE EXPRESSION RESPONSES OF PRIMARY HEPATOCYTES TO VARIOUS HEPATOTOXINS.

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Pharmaceutical companies are aggressively evaluating screening systems to shorten the time required to identify leads and simultaneously eliminate poor drug candidates. To this end, both *in vitro* cell culture systems and gene expression profiling are being widely used. Immortalized cell lines, while useful in certain situations, have not yielded the breadth of toxicological information required to aid decision making for lead optimization. Primary hepatocytes are currently being evaluated for use in toxicity screening. As a first step for determining the utility of expression profiling in primary hepatocytes, experiments were designed around "paradigm" compounds that elicit well-understood toxicities *in vivo*. Primary rat hepatocytes were exposed to various hepatotoxins including allyl alcohol (AA), isoniazid (ISO), methotrexate (MTX), phenobarbital (PB), tacrine (TAC), Trovan (TRO), and valproic acid (VPA). Cells were exposed to each compound for multiple dose and exposure times in order to put the gene expression responses into perspective. AA had only modest effects on gene expression possibly due to induction of aldehyde dehydrogenase that detoxifies acrolein, the toxic metabolite of AA. ISO did not have consistent effects on gene expression and alterations were modest. MTX induced genes indicative of an oxidant response such as catalase and glutathione synthetase. PB was nearly without effect in these studies. TAC induced expression of glutathione synthetase in addition to genes involved in reducing oxidative stress. TRO induced several genes of unrelated function and the responses were highly dependent on exposure time. VPA induced several genes including those linked to peroxisome proliferation in a dose- and time-dependent manner. VPA treatment of rats *in vivo* is linked to peroxisome proliferation. Thus, hepatotoxin gene expression profiles derived from treatment of primary rat hepatocytes can provide insight into toxic mechanism.

1344 PREDICTIVE MODELING OF HEPATOTOXICANTS USING MICROARRAYS AND A LINEAR DISCRIMINANT MODELING APPROACH.

A. L. Castle, M. W. Porter, M. Elashoff, K. R. Johnson, H. W. Sun, M. S. Orr, C. G. Chang and D. L. Mendrick. *Toxicology, Gene Logic, Inc., Gaithersburg, MD.*

In order to increase speed and depth of toxicological screening we have established a toxicogenomic database, ToxExpress™, that can be used to accurately predict and help classify potential toxicants using gene expression changes. Using the Affymetrix GeneChip® RGU_34 series microarray, we have collected expression information from over 1, 000 rat liver samples for greater than 26, 000 microarray elements per sample. Rats were treated with known hepatotoxicants that exert pathologies such as necrosis, steatosis, cholestasis, and hepatitis along with appropriate controls. In general, samples from rats treated with vehicle, pharmacologic, or toxic doses of each compound were collected at three or more time points. Over 500 general marker elements and more than 1, 000 compound/class-specific markers were found to be predictive based on discriminative ability and determined to be significant using randomization procedures. Predictive models were built using linear discriminant analysis (LDA). Preliminary results show successful predictions of toxicity using the markers determined to be discriminative, as well as for subsets of these markers. In addition, more specific classification of toxic compounds was achieved with compound- and class-specific markers. We are continuing efforts to validate our liver models, expand them into other organ systems and cell types, and investigate the mechanisms of action of marker genes.

1345 PREDICTING TOXICITY IN TWO DISTINCT SECTIONS OF THE KIDNEY *VIA* MICROARRAY ANALYSIS.

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In the toxicogenomics field, many debates have centered on whether injury to a specific area of the kidney can be detected by gene expression profiling of the entire organ. The feasibility of detecting toxicity in the kidney was evaluated by exposing

Sprague-Dawley rats for 6 hrs, 24 hrs, and 7 days to multiple doses of either puromycin aminonucleoside (PAN), which induces glomerular injury, or cisplatin, which produces proximal tubular injury. Clinical chemistry and histopathologic examination indicated clear evidence of overt toxicity only after 7 days of exposure unlike the 6 and 24 hour time points that failed to exhibit any signs of damage. The kidneys from all time points and doses were analyzed by the Affymetrix RGU_34 GeneChip®. A subgroup of genes selected from statistically significant expression differences induced by PAN-treated rats from both the 6 and 24 hour times were used to develop a predictive model, as we were interested in determining if acute expression changes can predict late events. The model was tested against itself as well as against the cisplatin-treated samples in order to determine if it could predict the samples as toxic or non-toxic. The results indicate that the PAN model was accurate in predicting itself. More importantly, the PAN model was accurate in predicting the cisplatin-exposed samples, providing evidence that expression profiling can be used in predictive toxicology studies even if a complex tissue is analyzed and the damage occurs in a specific area of the organ. Gene expression alterations are present before overt toxicity is observed by classical parameters of toxicity. This suggests that expression profiling is a more sensitive measure of toxicity that is capable of predicting the manifestations of a toxic phenotype much earlier than classical techniques.

1346 GENE EXPRESSION IN SPLEENS OF RATS DOSED WITH ESTRADIOL, HYDROXYUREA, OR TAMOXIFEN.

G. M. Farris. *Database, PHASE-1 Molecular Toxicology, Santa Fe, NM.*

The differential gene expression in spleens of rats dosed with immunotoxicants were evaluated to determine patterns related to tissue damage and mechanisms of toxicity. Rats received an intraperitoneal injection of estradiol, hydroxyurea or tamoxifen at two concentrations and then tissues were collected at 6, 24 and 72 hours later. Estradiol and tamoxifen induced lymphocyte necrosis in spleen follicles at 72 hours and hydroxyurea induced mild lymphocyte hyperplasia. RNA from spleen, liver, and kidney samples were hybridized on the PHASE-1 700-gene rat toxicology array. Comparison of hydroxyurea-induced gene expression in the spleen at 72 h demonstrated a strong similarity with estradiol gene response after 6 h. The stimulation of corticosterone production by hydroxyurea as described in the literature could be inducing this spleen response. Our evaluation of gene expression includes comparisons with other steroids including dexamethasone and prednisone and comparisons in spleen, liver, and kidney. Differential gene expression can provide information about mechanisms of toxicity with a simple screening protocol when a gene expression database including traditional toxicology data is used for comparisons.

1347 EVALUATION OF TUMOR-PROMOTING ACTIVITY BY GENE EXPRESSION PROFILING.

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The number of potential leads pharmaceutical companies obtain has increased steeply in the last years due to advances in combinatorial chemistry and high throughput screening. We therefore develop our predictive toxicology platform that will help pharmaceutical companies to focus as early as possible on the most promising candidates by excluding potentially harmful lead compounds. Carcinogenic activity may be a knock-out criterion for a drug. While genotoxic activity can be detected in short-term assays (eg Ames test), the detection of carcinogenicity by tumor promoting compounds represents a real bottleneck since long-term animal studies are necessary. Using our broad gene expression profiling technology platform we have analyzed liver samples of rats fed with a panel of tumor promotors of different mode of action-classes (eg Phenobarbital, WY14, 643, Ethinylestradiol) and compounds with cytotoxic activity (Furan, CCl_4). The two pairs of 2, 4- and 2, 6-diamino- and dinitrotoluenes that have or do not have tumor promoting activity, respectively, were also included in our analysis. We have conducted our experiments in two phases. In phase 1 we identified marker genes (regulated in response to a certain compound class) using Massive Parallel Signature Sequencing (MPSS), Restriction-Mediated Differential Display (RMDD) and "discovery" DNA arrays. These marker genes were added to our TOXaminer DNA array containing several hundred cDNA fragments of toxicology-relevant genes. In phase 2 all samples were analyzed on this "grown" TOXaminer array. With our analysis we identified many genes known but also around 200 genes not known to be regulated by these substances. Using hierarchical clustering and principal component analysis we could group compounds according to their mode of action. Thus, our study shows that gene expression changes induced by a certain chemical is characteristic of that chemical and that compounds with similar mode of action induce similar gene expression changes.

1348 CROSS COMPOUND PREDICTIONS USING GENE EXPRESSION PROFILES FROM ACETAMINOPHEN OR CARBON TETRACHLORIDE, TWO STRUCTURALLY DISTINCT LIVER TOXICANTS.

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It is essential to develop tools to rapidly determine if new chemical entities (NCEs) have the potential to elicit a toxic response in critical organs such as the liver or kidney. In order to address this need, Gene Logic has developed the ToxExpress™ reference database as a pre-clinical tool for ranking NCEs. Two classical liver toxicants, acetaminophen (APAP) and carbon tetrachloride (CCl_4), were studied to determine if gene expression differences, measured by the Affymetrix RGU_34 GeneChip®, induced by a drug could predict toxicity in samples exposed to a solvent such as CCl_4. In this study, Sprague-Dawley rats were treated with either APAP or CCl_4 and liver samples were collected from multiple time points with replicate numbers of at least three. Elevations of ALT levels and signs of necrosis by histopathological examination confirmed that both APAP and CCl_4 induced liver damage at 24 hrs. A statistically significant subgroup of genes was selected from the gene expression alterations induced by the APAP-treated rats at 3, 6 and 24 hr time points. These genes were used to develop a predictive model. The model was tested against itself as well as CCl_4 in order to determine the capacity of the model to predict samples as toxic or non-toxic. The APAP model was accurate in predicting itself and all the 3, 6, 24, and 48 hr CCl_4-treated samples correctly. Interestingly, the 1 hr and 72 hr CCl_4-exposed samples were predicted as non-toxic. These samples failed to show evidence of necrosis and steatosis, and 72 hr samples displayed features of fibrosis and regeneration. This study provides evidence that predictions between structurally distinct toxicants are feasible.

1349 DOSE-RESPONSE BEHAVIOR OF ANDROGENIC AND ANTIANDROGENIC CHEMICALS: IMPLICATIONS FOR LOW-DOSE EXTRAPOLATION AND CUMULATIVE TOXICITY.

L. E. Gray, C. J. Wolf, J. Furr, M. G. Price, C. Lambright, V. S. Wilson and J. Ostby. *EB, USEPA, Research Triangle Park, NC.*

Concern has arisen regarding the shape of the Dose-Response curves in the low dose range for EDCs. Although it is generally assumed that noncancer effects, including those induced by EDCs, display a threshold, several scientists have questioned the validity of this assumption. In regards to cumulative risk, it is generally assumed that dose-additivity prevails for chemicals that act *via* a common mechanism of action. A TEF (Toxic Equivalent Factor) approach for mixtures is valid if the dose-response curves for all the chemicals are parallel. This presentation will examine the dose-response behavior of several androgenic and antiandrogenic chemicals. *in vitro*, antiandrogenic chemicals like hydroxyflutamide and M2, a vinclozolin metabolite, display antiandrogenic activity at low concentrations and AR agonist activity at high concentrations. U- or inverted U-shaped Dose-Response curves have been described *in vivo* for AR agonists. In addition, many AR-mediated *in vivo* and *in vitro* effects do not display obvious thresholds. Furthermore, the dose-response curves for the antiandrogenic developmental effects produced by vinclozolin (V) administration reveals that the responses to this AR antagonist do not display a common shape or ED50. When V and procymidone (P, an AR antagonist) are coadministered, the effects all appear dose-additive, suggesting that a single TEF could be used to assess cumulative toxicity. While cumulative developmental effects also were observed with coadministration of P and an inhibitor of fetal testosterone synthesis (di-n-butyl phthalate), it is evident that single TEF will not suffice because the relative potencies of these two chemicals varies from tissue to tissue. Our data indicate that cumulative adverse effects can be observed after coadministration of chemicals that affect the differentiation of the reproductive tract, regardless of the mechanism of action. Disclaimer: This is an abstract of a proposed presentation and does not necessarily reflect USEPA policy.

1350 EVALUATION OF THE 15-DAY INTACT MALE ASSAY: CAPABILITY TO DETECT THE ANTI-ANDROGEN FLUTAMIDE.

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The Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) recommended that the 15-day intact male assay be evaluated as a possible alternate Tier I screening assay to detect endocrine–active compounds (EACs). While many EACs have been tested in this assay, studies to address interlaboratory variation and route of exposure are warranted. To this end, male CD rats (n=15/group) were exposed to the anti-androgen flutamide (0, 0.5, 1, 10 or 50 mg/kg/day) by gavage for

15 days, then euthanized 2 hours after the final dose. Reproductive organ, accessory sex gland and thyroid weights were measured to detect endocrine effects. At 50 mg/kg/day, flutamide significantly reduced terminal body weight by 9% and increased relative liver weight by 17%. At both 10 and 50 mg/kg/day, there was a dose-dependent decrease in several organ weights, including 16 and 27% decreases in absolute epididymal weights, 21 and 48% decreases in relative prostate weights, and 27 and 61% decreases in relative seminal vesicle weights. The body weight decreases exhibited by the high dose animals were not sufficient to account for these organ weight changes. The decreases in these organ weights were similar in magnitude to previous results with the intact male assay (O'Connor et al., 1998), wherein 20 mg/kg/day flutamide given intraperitoneally decreased epididymal, prostate and seminal vesicles weights by 22, 44 and 55%, respectively. Testes and thyroid weights were not altered in either study. These data show that the 15-day intact male assay can reproducibly detect the endocrine activity of flutamide across different laboratories and routes of exposure.

1351 HERSHBERGER ASSAY: DOSE-RESPONSE AND COMPARISON OF TISSUE RESPONSES TO ANDROGENS GIVEN ORALLY AND SUBCUTANEOUSLY.

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Three experiments (Exp.) tested effects of androgen agonists testosterone propionate (TP) and methyl testosterone (MT) on male sex accessory tissues. The protocols were adapted from the OECD program for validation of the rodent Hershberger Assay. TP (s.c.; Exp 1) at doses of 0.1, 0.2, 0.4, 0.8, and 1.6 mg/kg/day (n = 6/group) or MT by gavage (p. o.) at doses of 0.1, 5, 10, 25 and 50 mg/kg/day (n = 6/group; Exp 2) was administered once daily for 10 days to 8-week old Sprague-Dawley rats (castrated postnatal day (pnd) 42-43, treated pnd 60-69). Exp. 3 evaluated the androgen antagonist flutamide (FT; 1, 5 or 10 mg/kg/day) in combination with MT (5 or 10 mg/kg/day). FT was administered p.o. first (ca. 8am) and MT was given p.o. ca. 4 hours later. Vehicle dosed controls were included in all studies. Rats were euthanized 24 hours after the final dose. The liver, glans penis (GP), ventral prostate (VP) (both fresh and fixed), seminal vesicles plus coagulating glands with contents (SVCG), Cowper's glands (CG), and levator ani and bulbo-cavernosus muscles (LABC) were weighed to the nearest 0.1 mg. TP produced distinct dose-responses in these tissues: (dose level to produce maximum tissue weight: wt.): GP at 0.4 mg/kg/day; VP and CG at 0.8 mg/kg/day; LABC at 1.6 mg/kg/day; and SVCG response was linear over the dose range. Responses (fold increase in tissue wt. compared to control) at TP 1.6 mg/kg/day ranged from ca. 2 to 25 in the order GP<LABC<CG<VP<SVCG. MT p.o. was somewhat less potent (shallower dose responses): fold increase in tissue wt. compared to control at MT 50 mg/kg/day ranged from ca. 1.6 to 10.6 in the order GP<LABC<CG<SVCG<VP, and Coefficients of Variance of tissue wt. ranged from 2 to 51, GP<LABC<SVCG, CG<VP. Agonist activity of MT was reduced by combined treatment with FT. No major differences in Dose-Response curves were seen between VP fresh or fixed. This comparison of assay performance by the relevant oral route to the s.c. route supports additional validation by both routes.

1353 DETECTION OF ENDOCRINE EFFECTS: AN ENHANCED OECD TEST GUIDELINE 407: STUDY WITH TAMOXIFEN.

F. Chuzel, G. Espuna, E. Barale-Thomas, C. Pallen and R. Bar. *Experimental Toxicology, Aventis CropScience, Sophia-Antipolis, France.* Sponsor: N. Carmichael.

As part of a global OECD exercise, an enhanced version of the OECD TG 407 (28-day rat toxicity study) is being evaluated to detect endocrine disrupters. A range of different molecules acting *via* different modes of action are included in this exercise, the effects of a nonsteroidal antiestrogen (tamoxifen) in male and female rats are reported here. Wistar rats were administered tamoxifen by gavage at 0, 5, 30 and 200 µg/kg/day for at least 28 days. Endocrine-related parameters included in the study were: necropsy of females in dioestrus only; assessment of sperm parameters (number, motility and morphology); determination of plasma hormone levels (T4, T3, TSH, LH, testosterone and estradiol). Significant decreases in body weight gain were observed in high dose males and females. Males treated at 200 and 30 µg/kg/day showed decreased secretion in prostate and seminal vesicles without apparent effects on sperm parameters. In females, tamoxifen induced interstitial gland hypertrophy in the ovaries, diffuse epithelial and glandular hyperplasia/hypertrophy in the uterus, and diffuse mucoid metaplasia in the vagina at 200 and 30 µg/kg/day. In addition, acinar atrophy of mammary glands were noted in both males and females at 200 and 30 µg/kg/day. No apparent effects were detected on thyroid hormone levels. These results support that a potent antiestrogen can be detected in the enhanced version of the OECD TG 407mainly through histopathological examination. (supported by EMSG and OECD)

1354 DETECTION OF ENDOCRINE EFFECTS: AN ENHANCED OECD TEST GUIDELINE 407; STUDY WITH METHYLTESTOSTERONE.

G. Espuna, F. Chuzel, G. Pohlmeyer Esch, C. Pallen and R. Bars. *Experimental Toxicology, Aventis CropScience, Sophia-Antipolis, France.* Sponsor: N. Carmichael.

As part of a global OECD exercise, an enhanced version of the OECD TG 407 (28-day rat toxicity study) is being evaluated for detection of endocrine disrupters. A range of different molecules acting *via* different modes of action are included in the exercise; the effects of an androgen agonist (methyltestosterone) in male and female rats are reported here. Wistar rats were administered methyltestosterone by gavage at 0, 10, 40 or 200 mg/kg/day in males and 0, 10, 100 or 600 mg/kg/day in females for at least 28 days. Endocrine-related parameters included in the study were : necropsy of females in dioestrus only; assessment of sperm parameters (number, motility and morphology); determination of plasma hormone levels (T4, T3, TSH, LH, testosterone and estradiol). A significant decrease in body weight gain (BWG) was observed in high dose males whereas all female treated groups showed a marked increase in BWG. Males at 40 and 200 mg/kg/day showed decreased testis weights and a low number of Leydig cells with diffuse degeneration of germinal epithelium associated with an alteration in the sperm parameters. In addition, prostate weights were increased in high dose males only. Females at 100 and 600 mg/kg/day showed gonadal atrophy (interstitial cells atrophy, follicular atresia) with epithelium hyperplasia of the uterus and vagina. Histopathological findings in both males and females were also observed in the liver, thyroid, adrenals, mammary glands and kidneys. Hormonal measurements showed increased TSH and decreased LH plasma levels in both sexes. These results support that a potent androgen can be detected in the enhanced version of the OECD TG 407. (Supported by EMSG and OECD)

1355 ASSESSMENT OF A FATHEAD MINNOW REPRODUCTION ASSAY FOR IDENTIFYING ENDOCRINE-DISRUPTING CHEMICALS WITH DIVERSE MODES OF ACTION.

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The USEPA has developed a short-term reproduction test with the fathead minnow to identify potential endocrine disrupting chemicals (EDCs). The assay is initiated by collecting baseline spawning data from reproductively-active adult fathead minnows for 21 d, followed by a 21 d exposure to sublethal concentrations of test material. Measurements are made of fecundity, fertility, hatch success, secondary sex characteristics, gonadal condition (e.g., histopathology), plasma vitellogenin, and plasma sex steroids (estradiol, testosterone, and 11-ketotestosterone). To evaluate performance of the assay, we have conducted tests with several chemicals representative of major modes of action of concern from an EDC perspective. Chemicals tested have included: methoxychlor (metabolites are estrogen receptor agonists); ZM 189, 154 (estrogen receptor antagonist); methyltestosterone and trenbolone (androgen receptor agonists); flutamide and vinclozolin (androgen receptor antagonists); and fadrozole (cytochrome P450 aromatase [CYP19] inhibitor). Reductions in fecundity were clearly evident in exposures to methoxychlor, methyltestosterone, trenbolone, flutamide and fadrozole. These reproductive effects were accompanied by changes in other parameters reflective of the expected mode of action. For example, in the fadrozole test, estradiol and vitellogenin levels were decreased in females, as would be predicted if CYP19 was inhibited. Likewise exposure to the androgen receptor agonists caused masculinization in females, while estrogenic chemicals produced feminization (e.g., vitellogenin production) in males. This assessment of the test method shows the utility of the assay to provide guidance in identifying diverse modes of action of potential EDCs. *This abstract does not necessarily reflect EPA policy.*

1356 DEVELOPMENT OF AN AMPHIBIAN-BASED ASSAY TO SCREEN FOR CHEMICAL EFFECTS ON THE THYROID AXIS.

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In response to the initial EDSTAC recommendations, research was conducted on the development of a *Xenopus laevis* based tail resorption assay for evaluating thyroid axis disruption. The results of these initial experiments highlighted key limitations associated with relying on tail resorption as a measure of anti/thyroid activity. The most critical being that the tail tissue of tadpoles in metamorphic climax are insensitive to perturbation by agonists and antagonist. To begin to improve on the initial proposal we have conducted experiment comparing the sensitivity of pre-metamorphic (stage 51) and pro-metamorphic (stage 54) tadpoles to the model

thyroid axis antagonists methimazole (control, 6.25, 12.5, 25, 50, 100 mg/L) and 6-propylthiouracil (control, 1.25, 2.5, 5, 10, and 20 mg/L). Tadpoles were exposed for a 2 wk. period, and developmental stage, thyroid size, and thyroid histology were examined at 1 and 2 wks after exposure. Methimazole treatment resulted in a dose dependent inhibition of metamorphosis at both developmental stages within one week, while 6-propylthiouracil treatment resulted in metamorphic inhibition in stage 51 tadpoles within one week and of both stages within two weeks. Further, both compounds caused dose dependent changes in thyroid gland morphology. These changes were characterized as reduced colloid, glandular hypertrophy, and cellular hyperplasia. Treatment failed to affect growth, even in tadpoles which experienced significant metamorphic inhibition. As determined from these endpoints, there were only minor differences in sensitivity observed among the 2 stages examined. These results indicate that tadpoles in the early stages of metamorphosis are sensitive to thyroid axis inhibition and that development of a short term, diagnostic amphibian-based thyroid screening assay shows considerable promise. *This abstract does not necessarily reflect EPA policy*

1357 INTERPRETING THYROID TOXICITY FOR RISK ASSESSMENT.

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Thyroid hormone is essential for normal brain development. A large number of chemicals, of both natural and synthetic origin, are known to affect thyroid function. Therefore, it is important to characterize the effects of specific chemicals on the ability of thyroid hormone to exert its action(s) during brain development. However, the present strategy for testing thyroid toxicity in rodent models employs end points of thyroid function only – chemicals are not tested for their ability to interfere with thyroid hormone action. This strategy is valid if and only if chemicals interfere with thyroid hormone action exclusively by affecting thyroid function, or that measurable changes in thyroid function are always associated with changes in thyroid hormone action. Considering that the consequences of thyroid hormone deficits during development are not remediable, and that thyroid hormone action is quite pleiotropic, end points of thyroid hormone action must be recruited into studies to be used for risk assessment. However, these measures must be identified and validated.

1358 EVALUATION OF A CANNULATION MODEL AND ISOFLURANE ANAESTHESIA FOR BLOOD SAMPLING IN THE RAT: EFFECTS ON SERUM PROLACTIN AND CORTICOSTERONE.

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The purpose of this study was to validate two different techniques for blood sampling in female Wistar Hannover rats, i.e. by permanent cannulation of the vena femoralis and by orbital puncture after isoflurane anaesthesia. To this purpose the effects of stress caused by the two techniques on two stress-sensitive hormones were measured, i.e. prolactin and corticosterone. Decapitation was used as a reference technique as it is believed to have minimal effects on these hormones. It is regarded by the American Veterinary Medical Association as an acceptable technique when properly performed (2000 report). Blood was sampled in all rats 7 days after surgery of the cannulated animals over a period of 4 hours at 4 timepoints. Repeated blood sampling was performed in ten rats while with the two other techniques six animals were used on each timepoint. No in-life technical problems were encountered with any of these techniques although in cannulated animals the amount of blood that can be sampled is limited and sometimes insufficient. Both isoflurane anaesthesia and cannulation induced higher prolactin levels than decapitation and isoflurane anaesthesia induced higher prolactin levels than cannulation. Isoflurane anaesthesia and decapitation were comparable when corticosterone levels were measured. Cannulation induced higher corticosterone levels than both other techniques. Cannulation and isoflurane anaesthesia are technically feasible but have the potential to induce alterations in the levels of one or both hormones. Decapitation does not alter the levels of prolactin and corticosterone. Cannulation offers the possibility of repeated blood sampling in the same animal which reduces the inter-animal variability which can be useful for e.g. pharmacokinetic studies and PK/PD studies.

1359 NADPH OXIDASE *VERSUS* MITOCHONDRIAL OXIDANT STRESS IN ACETAMINOPHEN TOXICITY.

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Previous studies have demonstrated that nitrotyrosine (NT) adducts in proteins are formed in the centrilobular regions of the livers of mice dosed with acetaminophen (APAP). NT is formed by peroxynitrite, a species produced by the very rapid reaction of superoxide and nitric oxide. Previous studies indicated that pretreatment of mice or rats with macrophage inactivators resulted in decreased toxicity and NT formation. The initial site of nitration was hepatic sinusoids. These data suggested a role for activated Kupffer cells in peroxynitrite formation. In the present study, we investigated the importance of superoxide generated by NADPH oxidase (present in Kupffer cells and neutrophils) in the development of APAP hepatotoxicity. APAP toxicity was compared in genetically engineered (knock out, KO) mice deficient in the gene encoding gp91phox, a large critical subunit of NADPH oxidase, and C57BL/6J (wild type, WT) mice. Intracellular sources of superoxide (ie., mitochondria) were evaluated by measurement of glutathione disulfide (GSSG) in hepatic mitochondria of KO and WT mice. No significant differences were found between KO and WT mice in hepatotoxicity. Liver glutathione was depleted by > 88% at 1 hour in both KO and WT mice. The mitochondrial GSSG-to-GSH was increased by 6 fold at 1 hour and by 7 fold at 2 hours in both KO and WT mice. Immunohistochemical analyses indicated a centrilobular distribution of APAP protein adducts and NT in both groups of mice. Sinusoidal staining for APAP and NT was present at 1 hour and subsequently hepatocyte staining for APAP and NT was observed in both groups of mice. These data indicate that superoxide from NADPH oxidase does not significantly contribute to peroxynitrite formation in APAP toxicity. However, peroxynitrite formation in sinusoidal endothelial cells and mitochondrial oxidant stress are important early events in acetaminophen toxicity.

1360 MEMBRANE ASSOCIATION OF MURINE GSTA4-4, A GLUTATHIONE TRANSFERASE THAT ACTS ON LIPID PEROXIDATION PRODUCTS.

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Reactive oxygen species (ROS) react with proteins, nucleic acids, and lipids. The latter process produces lipid hydroperoxides and electrophilic α, β-unsaturated aldehydes including 4-hydroxynonenal (4-HNE). The conjugation of 4-HNE with glutathione is catalyzed by specialized alpha-class GSTs exemplified by murine mGSTA4-4. The crystal structure of mGSTA4-4 reveals the presence of two highly exposed lysine residues (K115, one per subunit). Position 115 is occupied by acidic residues in most other alpha-class GSTs. We hypothesized that the positive charges of K115 residues in mGSTA4-4 may interact with negative charges on membranes. This could "dock" the enzyme to membranes, *i.e.*, the site of generation of its substrates (lipid peroxidation products). Therefore, we examined the intracellular localization of mGSTA4-4 in hepatocytes of normal mouse liver and in transfected HepG2 cells by fluorescence microscopy and digital deconvolution. mGSTA4-4 was found to be predominantly localized at or near the plasma membrane in transfected HepG2 cells, as well as in hepatocytes where it is expressed endogenously. *in vitro*, mGSTA4-4 associated with liposomes, and this interaction was potentiated when the liposomes contained phosphatidylserine. Mutating lysine-115 to glutamic acid resulted both in a loss of the plasma membrane targeting of mGSTA4-4 and in a significant reduction of its binding to liposomes *in vitro*. These data suggest that lysine-115 is critically important for the membrane association of mGSTA4-4, most likely by entering into an electrostatic interaction with negatively charged phospholipid headgroups, including phosphatidylserine. Membrane association of mGSTA4-4 may facilitate the transfer of lipid peroxidation products from the membrane to the active sites of the enzyme.

1361 GENERATION AND CHARACTERIZATION OF A KNOCKOUT MOUSE LACKING GSTA4-4, A GLUTATHIONE TRANSFERASE THAT METABOLIZES LIPID PEROXIDATION PRODUCTS.

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Lipid peroxidation products have signaling functions and, at higher concentrations, are toxic and may trigger cell death. The compounds are metabolized predominantly by glutathione S-transferases exemplified by mGSTA4-4, an enzyme highly efficient in glutathione conjugation of 4-hydroxyalkenals, and possessing glutathione peroxidase activity toward phospholipid hydroperoxides. Metabolism of lipid peroxidation products may thus have a dual role: signal termination and detoxification. To determine the physiological implications of these processes, we generated a mGSTA4-4 knockout mouse by homologous recombination. The mouse is viable, although preliminary observations on a limited number of available animals suggest lower fertility. Northern and Western blotting confirmed the

absence of *mGSTA4* mRNA and mGSTA4-4 protein in tissues of mice homozygous for the gene disruption. However, residual 4-HNE-conjugating activity was measurable, indicating the presence, and perhaps compensatory upregulation, of alternative enzymes catalyzing that reaction. The activity was 22% of wild-type in lung, 42% in liver, and 35% in brain. Because of the significantly lower capacity for 4-HNE metabolism, the knockout mouse will be a useful experimental model for the elucidation of toxic effects of lipid peroxidation products.

1362 CYTOPROTECTION FOLLOWING THE INDUCTION OF ENDOPLASMIC RETICULUM STRESS PROTEINS IS CELL TYPE AND TOXIN DEPENDENT.

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Recent evidence suggests that induction of endoplasmic reticulum (ER) stress proteins increases cellular tolerance to agents which cause cytotoxicity through either oxidative stress or covalent binding. Previous work has been done primarily in kidney and neuronal cell types. To determine if protection against cytotoxic agents following the induction of endoplasmic reticulum (ER) stress proteins is a phenomenon that can be generalized across cell types, we assessed the protective effect of an ER stress response in a human hepatocyte cell line (HepG2), a rat hepatocyte cell line (H4IIE), a porcine kidney cell line (LLC-PK1), and a human lymphocyte cell line (K562). The ER stress response was induced by pretreatment with the glycosolation inhibitor, tunicamycin. Induction of the ER stress proteins GRP78, GRP94 and protein disulfide isomerase (PDI) was assessed by immunoblotting. In all cells, at least two of the ER stress proteins were induced, although the pattern varied between cell types. Tolerance to 3 hour challenges with the alkylating agent iodoacetamide (IDAM), the oxidative stressor *tert*-butylhydrogenperoxide (TBHP), the redox cyling agent menadione, or a 2 hour challenge with the reactive intermediate of acetaminophen NAPQI was assessed by the MTT assay 24 hours after addition of the toxin. In LLC-PK1 cells, the dose of toxin resulting in 50% survival increased for IDAM (505% ± 115%) and TBHP (168% ± 58 %) as previously reported, and for menadione (117% ± 9.0%) and NAPQI (205% ± 45%). However, in the hepatocyte and lymphocyte cell lines, increased tolerance was observed only in the HepG2 cells, where after ER stress, cells were more tolerant only to IDAM, exhibiting an 69% ± 8.8% increase in the TD50 after tunicamycin treatment. Induction of ER stress proteins in H4IIE cells and K562 cells did not alter susceptibility to any toxins tested in those cells. Our results indicate that protection afforded by the induction of an ER stress response is dependent on the cell type and toxin.

1363 ENGINEERING OF A BINARY TRANSGENIC MOUSE BROADLY EXPRESSING FPG FOR TESTING THE PATHOLOGICAL ROLE OF OXIDATIVE DNA DAMAGE.

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Reactive oxygen species (ROS) are formed endogenously, enhanced by some xenobiotics, and are believed to promote a number of pathologic processes. ROS enhance the formation of 8-oxoguanine (8-oxo-G), a promutagenic oxidative DNA lesion that undergoes base excision repair in mammals *via* oxoguanine glycosylase (OGG1). OGG1's bacterial counterpart is formamidopyrimidine DNA glycosylase (fpg). FPG exhibits an 80-fold higher activity and a broader substrate specificity compared to OGG1. To determine the role that oxidative DNA damage and repair plays in ROS-mediated toxicity, we engineered tetracycline-regulable transgenic mice that broadly expressed fpg during development. To optimize tet-regulated expression in these mice, trans-activator (beta-actin /CMV-IE promoter / tTA) and operator (TetO/fpg) constructs were simultaneously introduced into murine embryonic stem (ES) cells. The resulting ES clones were selected for those with optimal performance characteristics, which were used to construct murine lines. To more easily detect fpg *in vivo*, a FLAG epitope was fused in-frame with our fpg cDNA. In addition, TetO /beta-Geo was introduced into some ES cell clones to allow for lac-Z and neomycin detection in affected cells. Prior to the introduction into ES cells, constructs were tested in HEK 293 cells. In these cells carboxy-terminal FLAG-tagged fpg was correctly localized to the cell nucleus, although it exhibited lower repair activity (~50%) compared to our wild-type fpg clones. Co-transfection of actin/tTA strongly activated expression of both TetO/fpg-FLAG and TetO/beta-Geo as determined by Western blotting and lacZ cell staining. Transgene expression was abolished when cells were cultured in the presence of doxycycline. We are currently assessing the ROS response of our ES cell clones in response to various xenobiotic agents (Support: Canadian Institutes of Health Research).

1364 MUTATION OF THE TSC-2 GENE IN EKER RATS IS ASSOCIATED WITH REDUCED EXPRESSION OF 8-OXOGUANINE-DNA GLYSOSYLASE (OGG-1) AND A LACK OF INDUCIBILITY DURING OXIDATIVE STRESS.

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Treatment of either wild type (Tsc-$2^{+/+}$) Eker rats, or those carrying the mutant Tsc-2 allele (Tsc-$2^{EK/+}$) with 2, 3, 5-tris-(glutathion-S-yl)hydroquinone (TGHQ; 2.5 µmol/kg, i.p. for 4, 8 or 16 wks), a potent nephrotoxic metabolite of HQ, causes cell necrosis and proliferation within the outer stripe of the outer medulla (OSOM) in both groups of rats. However renal cell carcinomas only arise in the OSOM of the mutant Tsc-$2^{EK/+}$ strain. These findings provide compelling evidence that cell proliferation per se is not sufficient for tumor development. TGHQ can redox cycle and generate reactive oxygen species, and the mutation spectra induced by TGHQ following treatment and replication of the supF gene in both human AD293 cells and Eschericha coli MBL50 cells is consistent with that reported for OH•-induced mutations. 8-Oxo-deoxyguanosine (8-oxodG) is not only a measure of oxidative stress, but is a potentially mutagenic lesion. TGHQ (2.5 µmol/kg, i.p.; 5X/wk for 4, 8, 16 wks) increased 8-oxodG 2 fold within the OSOM of both rat strains at 4 wks. While elevated 8-oxodG levels were sustained in Tsc-$2^{EK/+}$ rats, they returned to baseline levels in Tsc-$2^{+/+}$ rats at 16 wks. Interestingly, although OGG-1 expression in Tsc-$2^{+/+}$ rats increased significantly during treatment with TGHQ, no such induction occurred in Tsc-$2^{EK/+}$ rats. Furthermore, the constitutive expression of OGG-1 was 3 fold lower in Tsc-$2^{EK/+}$ compared to Tsc-$2^{+/+}$ rats. These results suggest that mutation of the Tsc-2 gene is coupled to the suppression of constitutive OGG-1 expression and interferes with the ability to up-regulate this gene following oxidative stress. Moreover, the high basal and inducible OGG-1 levels in the wild-type Eker rats likely provide for a more efficient removal of oxidative DNA damage in these rats, thereby limiting tumor development. The basis for the lack of OGG-1 induction in Tsc-$2^{EK/+}$ rats is unknown and is currently being investigated. (GM39338, ES07784).

1365 COVALENT MODIFICATION OF PEPTIDES BY 4-HYDROXYNONENAL AND 4-OXONONENAL.

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Previous work has demonstrated that the α, β-unsaturated aldehydes 4-hydroxynonenal (4HNE) and 4-oxononenal (4ONE) are generated during lipid peroxidation. Recent evidence has implicated that covalent modification of proteins by 4HNE may play a role in some pathophysiological processes. Currently, the reactivity of 4ONE toward proteins is unknown. The purpose of this study was to identify residues that react with these α, β-unsaturated aldehydes, characterize the covalent adduct(s) formed, and measure the kinetics of adduction. Model peptides containing one or more nucleophilic residues (i.e. Cys, Met, Lys, Arg, and His) were reacted with a 20-fold excess of 4HNE and 4ONE at pH 7.4 and 37 °C. Control and treated peptides were analyzed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), and post-source decay (PSD) analysis was used to confirm the presence of a peptide adduct and identify the residue modified. Bimolecular rate constants for the reaction of glutathione (GSH) with 4HNE and 4ONE were measured. Peaks with mass corresponding to the peptide with a Michael adduct were observed in the mass spectra of model peptides containing either Cys, His, or Lys that had been treated with 4HNE. In contrast, peaks with mass corresponding to the peptide with a Michael adduct were observed in the mass spectra of model peptides containing either Cys, His, Lys or Arg that had been treated with 4ONE. The rate constants for the reaction of GSH with 4HNE and 4ONE were found to be 1.33 and 145 $M^{-1}s^{-1}$, respectively, indicating a >100-fold difference in potency for GSH modification between these two compounds. The results demonstrate that both 4HNE and 4ONE react with nucleophilic peptide residues to form covalent adducts but with contrasting specificity and a remarkable difference in potency for GSH conjugation. (Supported by grants NIH/NIAAA R01AA09300, NIH/NIEHS R01ES09410 and PHS 5T32AA07464-25.)

1366 OLTIPRAZ AND TERT-BUTYLHYDROQUINONE DO NOT INDUCE GLUTATHIONE S-TRANSFERASE IN A COMPLETELY IDENTICAL MANNER.

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Both oltipraz [4-methyl-5-(2-pyrazinyl)-3H-1, 2-dithiole-3-thione] and tert-butylhydroquinone (tBHQ) induce a similar range of drug metabolizing enzymes, a range that does not include most cytochrome P450s. Among the most prominent

responding enzymes is glutathione S-transferase (GST) and in the rat, the GSTA2 form has been the most studied. After transiently transfecting a luciferase reporter construct consisting of an ARE enhancer and a HNF1 binding region (-872 to -582) and the minimal promoter region (-164 to +1) of rGSTA2 into rat hepatoma cells (H4IIE), luciferase activity was induced to an order of magnitude greater extent by 500mM tBHQ than by 500mM oltipraz. With a reporter construct containing the XRE enhancer and HNF1 binding regions (-1110 to -812), and the minimal promoter region, luciferase activity was induced to a much lesser extent, but again tBHQ induced more than oltipraz (6-fold). With a reporter construct containing a large portion of the proximal 5' flanking region of the GSTA2 gene (-1651 to +1), tBHQ induced to a 20-fold greater extent than oltipraz and in a slightly more robust manner than with the ARE/HNF1-only containing construct. All of these luciferase responses contrasted with the induction of the rGSTA2 mRNA in H4IIE cells which was only slightly better induced (1.5-fold) by tBHQ compared to oltipraz. (In intact animals, the oltipraz response was far greater in magnitude than the response to tBHQ). rGSTA2 mRNA responses to both agents were similarly (completely) inhibited by 1 uM actinomycin D co-treatment. With cycloheximide, (1uM), the slightly smaller oltipraz response was completely inhibited but with tBHQ, some induction remained. Thus, despite many characteristics in common, oltipraz appears capable of up-regulating drug metabolizing enzymes in a manner different from tBHQ, and by mechanisms outside of XRE and ARE enhancers.

1367 INCREASED HEPATOTOXICITY OF ACETAMINOPHEN IN TUMOR NECROSIS FACTOR ALPHA (TNFα) R1 (P55) KNOCKOUT MICE IS ASSOCIATED WITH DECREASED ACTIVATOR PROTEIN-1 (AP-1) ACTIVATION.

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Tumor necrosis factor alpha (TNFα) is a macrophage-derived proinflammatory cytokine implicated in the hepatotoxicity of acetaminophen. In the present studies, we examined possible mechanisms mediating the actions of TNFα. Treatment of wild type control mice with acetaminophen (300 mg/kg, i.p.) resulted in a time-dependent induction of centrilobular hepatic necrosis and increased serum transaminase levels, which reached a maximum after 18 hours. Knockout mice lacking TNFR1 (p55) were found to be significantly more sensitive to acetaminophen than wild type mice, as determined histologically and by increases in serum transaminases. This was correlated with decreased levels of the antioxidants, superoxide dismutase and heme oxygenase, when compared to wild type mice. To determine if signaling pathways regulating the production of antioxidants were altered in TNFR1 knockout mice, we analyzed hepatic levels of c-Jun N-amino terminal kinases (JNKs) and its downstream target, AP-1. Western blot analysis revealed that administration of acetaminophen to wild type mice resulted in a time-dependent activation of JNK, which was evident within 3 hours. Nuclear binding activity of the transcription factor, AP-1, measured by gel shift assays, was also increased. These responses were significantly reduced in mice lacking TNFR1. These data suggest that TNFR1 plays an important role in the activation of JNK and AP-1, as well as in increases in antioxidants during acetaminophen-induced hepatotoxicity. Supported by NIH GM34310 and ES06897.

1368 INDUCIBLE NITRIC OXIDE SYNTHASE KNOCK-OUT (iNOS(-/-)) MICE EXHIBIT INCREASED OXIDATIVE STRESS INJURY IN RESPONSE TO ADRIAMYCIN (ADR)-INDUCED CARDIOTOXICITY.

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ADR is a chemotherapeutic agent known to cause dose-related cardiotoxicity. Heart injury associated with ADR treatment involves the generation of free radicals, such as superoxide. Increases in superoxide in the presence of reactive metals leads to generation of the injurious hydroxyl radical. Alternatively, superoxide can react with nitric oxide (NO) forming peroxynitrite. Therefore, the levels of NO can alter the fate of superoxide radicals *in vivo*. Here we investigate the role of NO *in vivo* in ADR-induced cardiotoxicity using an iNOS(-/-) mouse model. Electron microscopy demonstrates that 5 days following one intraperitoneal injection of ADR (20 mg/kg), iNOS(-/-) mice exhibit cytoplasmic swelling and degradation of mitochondria as compared to wildtype (iNOS(+/+)) mice. Tissue damage, assessed by creatine phosphokinase, lactate dehydrogenase, and cardiac troponin serum levels, is increased in iNOS(-/-) mice as compared to iNOS(+/+) mice, supporting the pathological findings. Antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase are increased to a larger degree in iNOS(+/+) mice than iNOS(-/-) mice following ADR, indicating a compromise in their ability to illicit an adaptive response to ADR-induced oxidative injury. The oxidized to reduced glutathione ratio increases in iNOS(-/-) mice treated with ADR and an increase in 4-hydroxynonenal (4-HNE) adducts is observed 24 hours following treatment, further verifying an increase in oxidative stress in the knock-out model. Time course studies indicate an increase in nitrotyrosine prior to the increase in 4-HNE in iNOS(+/+) mice, but not in iNOS(-/-) mice. Endothelial NOS protein levels are unchanged following treatment with ADR in both iNOS(-/-) and wildtype mice. These findings suggest that ADR-induced NO production is an early cardioprotective event associated with adaptation to oxidative stress.

1369 OXIDATIVE STRESS CAUSES THE FORMATION OF TWO DISULFIDES IN HUMAN THIOREDOXIN.

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Thioredoxin (Trx) is a small protein that restores disulfide bonds in proteins to the reduced form following oxidative stress. Trx contains two essential active site cysteines that cycle between the dithiol and disulfide forms as Trx reduces target proteins. Human Trx contains three additional cysteines, the functions of which are unclear. The purpose of the present study was to determine the redox state of human Trx under defined redox conditions *in vitro* and before and after oxidative stress within cells. Purified Trx was reduced with DTT or oxidized with diamide or hydrogen peroxide, derivatized with iodoacetic acid which carboxymethylated thiols but not disulfides, and separated by non-reducing, non-denaturing polyacrylamide gel electrophoresis. MALDI-TOF mass spectrometry of tryptic digests of individual gel bands demonstrated unequivocally the locations of carboxymethylated cysteines and disulfide bonds. Human Trx treated with dithiothreitol contained no disulfides. Trx treated with diamide or hydrogen peroxide contained two disulfide bonds: one between Cys32 and Cys35 (the active site cysteines), and one between Cys62 and Cys69. To define the midpoint potentials, Trx was equilibrated in glutathione redox buffers. The midpoint potential for the formation of the active site disulfide, determined by plotting the ratio of the oxidized and reduced forms of Trx against the potential of the redox buffer, was -230 mV. The second disulfide bond formed only under more oxidizing conditions, with a midpoint potential of about -150 mV. Within cells, the redox state of Trx was -280 mV, and the two-disulfide form of Trx was not detected. However, Trx was rapidly oxidized to the two-disulfide form upon exposure of the cells to t-butylhydroperoxide. Thus, human Trx contains a second dithiol that undergoes oxidation during oxidative stress. The function of this non-active site disulfide is unknown, but its position near the interface region suggests that it may regulate redox-dependent interactions with other proteins.

1370 INVOLVEMENT OF THE ELECTROPHILE RESPONSIVE ELEMENT (EPRE) IN THE ACTIVATION OF HEPATIC STELLATE CELLS DURING FIBROGENESIS.

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Hepatic stellate cells (HSC) produce the type I collagen in normal and cirrhotic liver. During liver fibrogenesis HSC undergo an activation process that includes increased proliferation and synthesis of extracellular matrix proteins, along with a phenotypic transformation to myofibrotic-like cells. Activated HSC contribute to chronic hepatic inflammation, which ultimately leads to cirrhosis. We have found a 20-fold increase of NAD(P)H: quinone oxidoreductase 1 (NQO1) enzymatic activity and protein levels in a HSC line derived from cirrhotic (CFSC) rat liver compared to that of normal (NFSC) rat liver. This NQO1 up-regulation appears to be responsible for the increased resistance of the CFSC line cells to menadione cytotoxicity. Gel mobility shift assays and functional analysis using transient transfection experiments suggest the involvement of the Electrophile Responsive Element (EPRE) in the up-regulation of the NQO1 gene expression in CFSC cells. In conclusion, the EPRE-signaling pathway, which regulates several antioxidant genes, may play a major role in the resistance of HSC against oxidative stress that occurs during hepatic fibrogenesis. This EPRE-mediated resistance may be part of the HSC activation process, which takes place during liver injury. [Supported by NIH R01 AA11885 & R01 AA09300-05].

1371 OLIGONUCLEOTIDE MICROARRAY ANALYSIS REVEALS AN ANTIOXIDANT RESPONSIVE ELEMENT-DRIVEN GENE SET INVOLVED IN CONFERRING PROTECTION FROM AN OXIDATIVE STRESS-INDUCED APOPTOSIS IN IMR-32 HUMAN NEUROBLASTOMA CELLS.

J. Li, J. M. Lee and J. A. Johnson. *UW School of Pharmacy, Madison, WI.* Sponsor: J. Johnson.

The current study was aimed to investigate whether and how tert-butylhydroquinone (tBHQ) prevents hydrogen peroxide-induced apoptosis in neuroblastoma cells. Hydrogen peroxide at a final concentration of 200 μM induced IMR-32 cells

to undergo apoptosis with morphological changes such as condensed and fragmented nuclei (TUNEL assay), increased caspase-3 activation, and poly ADP-ribose polymerase cleavage. In cytotoxicity assays and TUNEL assays, tBHQ pretreatment (10 μM) attenuated hydrogen peroxide-induced cell death and the number of TUNEL-positive cells, respectively. We hypothesize that tBHQ-mediated activation of the antioxidant response element (ARE) is critical for generating the protective response. Our laboratory has recently shown that a selective inhibitor of phosphatidylinositol 3-kinase (PI3-kinase), LY294002, blocks ARE activation by tBHQ. Addition of LY294002 30 min prior to tBHQ treatment completely reversed the protective effect of tBHQ. Oligonucleotide microarrays were used to analyze the gene expression profile associated with tBHQ treatment in the absence and presence of LY294002. 63 genes showed transcriptional upregulation by tBHQ. RT-PCR for selected genes also confirmed the gene expression pattern. Many of these genes function to combat oxidative stress and increase the cells detoxification potential through increased production of NADPH, glutathione (reduced form), and ATP. Some examples are NAD(P)H:quinone oxidoreductase, gamma-glutamylcysteine ligase regulatory subunit, thioredoxin reductase, glutathione reductase, and cytosolic malic enzymes. Inhibition of PI3- kinase significantly blocked the enhanced expression of 49 of the 63 genes induced by tBHQ. These are the first data to show the gene set involved in conferring protection from an oxidative stress-induced apoptosis and imply that the PI3-kinase-Nrf2-ARE pathway is critical in mediating protection from oxidative stress in human neuroblastoma cells.

1372 IS THERE A RELATIONSHIP BETWEEN URINARY AND LEUKOCYTE 8-HYDROXYDEOXYGUANOSINE IN HUMANS?

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8-Hydoxydeoxyguanosine (8-OHdG) is typically measured in DNA of white blood cells (WBCs) or in urine. Urine measurement is believed to provide an index of oxidative DNA damage repair. WBCs are considered a surrogate for target tissues. These markers are used almost interchangeably to assess the adverse effects of a variety of environmental factors. Only a few of the hundreds of studies assessing 8-OHdG as a biomarker of oxidative DNA damage in humans, have measured 8-OHdG in both urine and WBC DNA. Two recent studies reported non-significant inverse associations between urinary excretion of 8-OHdG and WBC DNA 8-OHdG. We examined this relationship in male roofers with (n=26) or without (n=12) exposure to polycyclic aromatic compounds and female dry cleaners and launderers with (N=18) or without (N=20) exposure to perchloroethylene. For males, urine and blood were obtained pre-shift at the start and post-shift at the end of a work week. For females, urine was obtained pre- and post-shift mid-work week, and blood was obtained once pre-shift mid-work week. 8-OHdG was measured by HPLC-ECD. Linear models were used to look for relationships between leukocyte DNA and urine levels of 8-OHdG. Terms for gender, exposure, smoking, and race were included. Interaction terms were also included to see if the relationship between blood and urine levels varied by these terms. The models were also used to estimate and test individual slopes. For the model comparing pre-shift urine with pre-shift blood, no main effects or interactions were statistically significant, but a significant inverse association (b = -0.1231, p = 0.0336) was noted for the individual sub-group of female controls. For the model comparing pre-shift blood with post shift urine no main effects or interactions or individual slopes were statistically significant. For the model comparing post-shift blood to post shift urine, the smoking interaction was significant (p = 0.0314). Results demonstrate a limited association between leukocyte DNA 8-OHdG and urinary excretion of 8-OHdG

1373 EFFECTIVE FACTORS ON URINARY 1-HYROXYPYRENE IN A KOREAN POPULATION.

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Urinary 1-hyroxypyrene (1-OHP) has been used as a biomarker for exposure to environmental carcinogenic polycyclic aromatic hydrocarbons (PAHs). For proper biological monitoring of the PAH exposure, associated factors with 1-OHP bioproduction should be clarified. In this study, we investigated effects of lifestyle, environmental factors, and genetic polymorphisms of metabolic enzymes, i.g., GSTM1, GSTT1, CYP1A1, and CYP1B1 on urinary 1-OHP levels in 700 Korean population (male, 65 % ; female, 35 % ; mean-age, 36 yrs, std, 10.79 yrs) who were not occupationally exposed to PAHs. Using questionnaire, we obtained information of cigarette smoking, indoor heating-system, housing-environment, consumption of well-done meat, vegetables, yogurts, coffee, alcohol, etc. We analyzed urinary 1-OHP and cotinine, a biomarker of smoking, by HPLC. To determine genotypes of the enzymes, we used PCR-RFLP and single base extension methods. As results, urinary 1-OHP was detected in 76 % of the subjects (range, 0.1-3.8 ug/L). In the Korean population, CYP1B1 codon 48 and 119 polymorphism were completely linked and CYP1B1 codon 48 polymorphism was associated with codon 432 polymorphism ($p < 0.05$). Urinary 1-OHP was correlated with urinary cotinine level, number of cigarette smoked before sampling, yogurts consumption, and GSTT1 polymorphism (Spearman Rank correlation, $p < 0.05$). However, polymorphisms of GSTM1, CYP1A1, and CYP1B1 were not associated with urinary 1-OHP levels. After multiple regression analysis, urinary 1-OHP was associated with only number of cigarette smoked before sampling ($p < 0.05$). In conclusion, recent cigarette smoking affects urinary 1-OHP compared to any other environmental, food- or genetic factors.

1374 NEUROPATHY TARGET ESTERASE (NTE) IN WHOLE BLOOD: BIOMARKER FOR EXPOSURE TO NEUROPATHIC ORGANOPHOSPHORUS COMPOUNDS (OPS).

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NTE is the target protein for neuropathic OPs that cause OP-induced delayed neurotoxicity (OPIDN). The inhibition/aging of brain NTE within hours of exposure predicts the potential for the development of OPIDN in susceptible animal models. Lymphocyte NTE has also found limited use as a biomarker of human exposure to neuropathic OPs. Recently, we developed a highly sensitive biosensor for NTE assay using a tyrosinase carbon-paste electrode to detect phenol produced by the hydrolysis of the substrate, phenyl valerate. The biosensor enabled NTE to be assayed in whole human and hen blood, whereas the usual colorimetric assay is impossible. NTE activity in hen and human blood was found to be 0.10 ± 0.03 and 0.19 ± 0.02 nmol/min x mg of protein, respectively. Mipafox I_{50} values for hen and human blood NTE were found to be 4.22 ± 0.12 and 6.27 ± 0.43 μM, respectively. To study the possibility of using blood NTE inhibition as a biochemical marker of neuropathic OPs exposure, NTE inhibition in brain and lymphocytes as well in brain and blood was studied 24 hr after dosing hens with the neuropathic OP, O, O-dipropyldichlorvinyl phosphate. Brain, lymphocyte and blood NTE were inhibited in a dose-responsive manner. There were strong correlations of NTE inhibition between brain and lymphocyte, brain and blood, and lymphocyte and blood. Taking into account the small sample volume required, simplicity of sample preparation, rapid analysis time, stability of samples after freezing, and strong correlation of NTE inhibition between blood and brain, our biosensor NTE assay for whole blood shows promise, not only as a biomarker of human exposure to neuropathic OP compounds, but as a predictor of OPIDN and an adjunct to its early diagnosis. Supported by ISTC, Project 1055.2.

1375 CHOLINESTERASE ACTIVITY IN MICE CHRONICALLY EXPOSED TO PYRIDOSTIGMINE BROMIDE.

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Pyridostigmine bromide (PB), given to soldiers during the Persian Gulf War as a prophylactic against potential nerve gas attack, is now thought to be a possible causative agent of the symptoms of Gulf War Syndrome. We have developed a murine model to test the effect of chronic exposure to PB on cholinesterase (ChE) activity in the blood and the brain. C57B/J mice were surgically implanted with osmotic mini-pumps to deliver a steady dose of PB over 7 days. ChE activity was determined by a modified version of the colorimetric assay of Ellman, et al. Total ChE activity was measured in diluted whole blood and brain homogenate samples. Blood acetylcholinesterase (AChE) activity was determined by inhibiting butyrylcholinesterase (BChE) with iso-OMPA (tetraisopropylpyrophosphoramide). BChE activity was then calculated by subtracting AChE activity from total ChE activity. Following 7 days of exposure to PB, reductions of blood ChE activity of 27%, 42%, and 55% were seen in the 3, 6 and 10 mg/kg/D dosage groups, respectively, when compared to pretreatment levels. The decrease in blood ChE activity, due primarily to a drop in AChE activity, indicated that the osmotic mini-pump delivery system was functioning properly. We found no change in CHE activity in sham-implanted control animals. Interestingly, while analysis of prefrontal cortex showed no significant change in AChE activity after exposure to PB, we observed a dose-dependent decrease in AChE activity (48% at 3 mg/kg) in the hypothalami of PB treated mice (see Ropp, et al. abstract, this meeting). We are currently evaluating ChE activity in other brain regions of these animals and other mice exposed to both chronic stress and PB. Our findings indicate that chronic exposure to PB pro-

duces a dose-dependent decrease in blood and hypothalamic cholinesterase activity possibly contributing to symptoms of Gulf War Syndrome. This research is funded by DoD contract DAMD17-00-C-0020.

1376 EVALUATION OF OXIDATIVE PROCESSES IN T LYMPHOCYTE PROLIFERATION AND DEATH WITH CYTOCHROME P450 INHIBITORS AND HIGH-SPEED, CAPILLARY GC/TOFMS MEASUREMENT OF VOLATILE COMPOUNDS.

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A murine T cell hybridoma (2B4.11) was used to investigate how oxidative processes contribute to cell proliferation and to cell death induced by various agents. Inhibitors of the oxygen-activating enzyme cytochrome P450 attenuated cell death caused by calcium ionophore or T cell receptor ligand, but not dexamethasone, at concentrations that did not cause cell death. These results suggest that the P450 monooxygenase system plays a role in some pathways that signal T cell death. In view of the role of cytochrome P450 and other pro-oxidant enzymes in lipid peroxidation, methodology was developed to identify and measure biomarkers of lipid peroxidation in T cells. Malondialdehyde, as judged by the spectrophotometric measurement of the thiobarbituric acid complex, was produced in T cells during proliferation, was enhanced in the presence of 1 mM dexamethasone, and was inhibited in the presence of 20 μM ascorbate. To monitor the volatile products of lipid peroxidation, petri dishes containing T cell hybridoma cells were incubated at 37 °C in a closed chamber equipped with a fan to disperse volatile products of cellular metabolism. The head-space gas was passed through a membrane (provided by Restek) to exclude water and was then collected and concentrated over a five-min period at –90 °C by a trap cooled by liquid nitrogen. Firing of the trap was achieved at 170 °C by application of a 90-volt current. Using this novel method, hexanal was easily identified by GC/TOFMS above the 10 mL of about 0.5×10^6 T cells/mL in RPMI 1640/10% fetal bovine serum. The simultaneous characterization of volatile products in a continuous assay provides a non-invasive approach to examining the kinetics of formation of volatile lipid peroxidation products formed during oxidative stress induced by pro-oxidant systems such as cytochrome P450. [Supported by Research Excellence Fund/Graduate School Research Support Fund (EMU) and NSF Grant DBI-9987063].

1377 CAN SERUM CALMODULIN BE USED AS A BIOLOGICAL MARKER IN CHRONIC LEAD EXPOSURE?

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We have evaluated whether serum calmodulin (CaM) can be used as a biological marker in chronic lead exposure. Thirty-six workers exposed to lead from a storage battery factory were selected as exposed group and twenty-six non-lead exposed workers were selected as control group. Confounding factors such as age, length of service, marriage and bad habits were carefully considered in the evaluation. Blood CaM, blood lead, calcium, magnesium, copper and zinc were determined from both lead and non-exposed workers and all data were analyzed using SAS software. The blood CaM was reduced in workers with high blood lead levels. At blood lead level higher or equal to 40 ug/dl; the blood CaM in exposed group was found to be (35.18 ± 6.92 ug/dl), which was significantly lower when compared to the control group (76.54 ± 10.35 ug/dl, $p<0.05$). Age, length of exposure, marriage status and bad habits had no effect on blood CaM levels ($p>0.05$). The correlation analysis results showed that blood lead and blood calcium was correlated to blood CaM levels ($p<0.05$), while blood magnesium, copper and zinc had no significant correlation with CaM ($p>0.05$). The results indicated that reduction of blood CaM in lead-exposed workers was accompanied by the increased blood lead. The concentration of 40 ug /dl might be considered as the biological threshold for blood lead, which coincided with the current diagnostic standard for blood lead in China. The results of the present study are promising and may indicate a role of CaM as a biological marker. However, further study with increased number of lead exposed individuals will be required before CaM could be suggested as a biological marker for chronic lead exposure.

1378 SIMULTANEOUS DETERMINATION OF N-7-HYDROXYETHYLGUANINE, O^6-HYDROXYETHYLGUANINE AND N-3-HYDROXYETHYLADENINE WITH LC/MS/MS.

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N-7-hydroxyethylguanine (7-HEG) has been previously described as the major DNA adduct *in vivo* after exposure to ethylene oxide (EO), with analysis by gas chromatography/mass spectrometry (GC/MS) or high performance liquid chromatography (HPLC) with fluorescence or electrochemical detection. Here we present a method for the determination of 7-HEG by HPLC and tandem mass spectrometry (LC/MS/MS). In addition, the method is also capable of analysis of O^6-hydroxyethylguanine (6-HEG) and N-3-hydroxyethyladenine (3-HEA) in one analytical run. For this purpose, DNA was isolated from mouse liver tissue by chloroform/phenol extraction. DNA samples were fortified with the adducts mentioned above. Subsequently, a neutral thermal hydrolysis was carried out. Separation of adducts from oligonucleotides was achieved by centrifugation of the hydrolysate using microconcentration filters. After injection and separation of the adducts employing a novel stationary phase (silica-bonded polyamide) for HPLC, the adducts were quantified by the use of their isotopically-labeled analogs N-7- and O^6-hydroxyethyl-[^{13}C-8, ^{15}N-7, 9]guanine (7-HEG* and 6-HEG*), and N-3-hydroxyethyl-[^{13}C-8, ^{15}N-6, 9]adenine (3-HEA*). Multiple reaction monitoring (MRM) was carried out to detect the fragments [M-44] for the analytes and their labeled internal standards. The limits of detection for the method were determined to be between 2.6 and 2.8 fmol on column depending on the particular adduct. The LC/MS/MS method presented here has advantageous over the previously published GC/MS methods, since derivatization of adducts could be omitted and all three major adducts of EO can be analyzed in one single analytical run.

1379 ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY (ESI-MS/MS) ANALYSIS OF 1-BROMOPROPANE MERCAPTURIC ACID METABOLITES IN URINE.

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1-Bromopropane (1-BP, CAS 106-95-5), an alternative solvent to chlorofluorocarbons, has been reported to cause reproductive and neurotoxicity in male rats. The related 2-bromopropane has been shown to cause similar toxicity in rats and amenorrhea, oligozoospermia, and anemia in workers. Activation to reactive intermediates may be important. Metabolism of 1-BP is complex, including debromination, CYP2E1 oxidation and glutathione S-conjugation. The formation of 3-bromopropionic acid and n-propanol as well as of S-n-propyl-glutathione, S-n-propyl-L-cysteine and the mercapturic acids N-acetyl-S-(n-propyl)-L-cysteine (M1), N-acetyl-S-(n-propyl)-L-cysteine-S-oxide (M2), N-acetyl-S-(2-carboxyethyl)-L-cysteine (M3), and N-acetyl-S-(3-hydroxy-n-propyl)-L-cysteine (M4). A potential biomonitoring method was developed to measure urinary levels of M1, M2, M3 and M4. Standards and a stable isotope-labeled analog of M1 as internal standard were synthesized using the general procedure of van Bladern et al. (1980). Samples mixed with internal standard were loaded onto Bond Elute C18 SPE columns. A fraction containing >90% of 1-BP metabolites was eluted with acetone for analysis by HPLC ESI-MS/MS on a 150X2 mm Phenomenex Aqua C18 column using a 10-min H2O:MeOH (1% acetic acid) linear gradient at 300 μL/min. The ESI-MS/MS operated in the positive ion mode to detect protonated M1, M2, M3 and M4 and used Selected Reaction Monitoring of major transition products. Urine samples fortified with standards were mixed with 10 μg/mL internal standard and evaluated for recovery, limits of detection and limits of quantitation. Calibration of M1, M2, M3 and M4 was linear from 30 - 10000 ng/mL ($r2>0.99$). Sample preparation and analysis appears to offer significant advantages over typical derivatization that would be required for GC-MS analysis of these compounds. Thus, 1-BP internal exposure levels for various exposure situations can be rapidly determined by analysis of these metabolites in a single assay using selective sample preparation.

1380 IMMUNOLOGICAL DETECTION OF HEXAMETHYLENE DIISOCYANATE ADDUCTS IN SERA FROM AUTOBODY SHOP WORKERS.

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Hexamethylene diisocyanate (HDI), a potent irritant and allergen, is widely used in the manufacture of polyurethanes for paints and coatings. Detection of HDI is problematic since diisocyanates are difficult to characterize, measure and control. Biomarkers would be of great benefit for early detection of exposure to prevent possible chronic adverse health effects. We have previously reported development of a sensitive and specific immunoassay for detection of HDI adducts on proteins. Microtiter plates coated with rabbit IgG from anti-HDI-keyhole limpet hemocyanin antiserum are used to capture HDI-adducted proteins. The immunoassay has a sensitivity of 95 nmol HDI adduct. To evaluate the potential use of the immunoassay to detect recent HDI exposure, sera were collected from six autobody shop workers who are exposed to HDI in paints. Serum samples were obtained from three of these workers periodically during one workday, and from the remaining three workers only at 24 hr after the start of the workday. Sera were also obtained from control non-exposed individuals. HDI adducts were detected using

peroxidase-labeled sheep anti-human albumin, followed by substrate. No HDI adducts were detected in any of the samples from control individuals nor in samples taken at the start of the workday. However, in one worker 99 nmol HDI adduct was detected at 4 hr, and 423 nmol was detected at 24 hr. HDI adducts were detected in sera from three of the individuals at 24 hr. Concentrations ranged from 104- 426 nmol adduct. These results indicate that this immunoassay has appropriate sensitivity to detect recent HDI exposure and can be applied for human biomonitoring of HDI in the workplace. Supported by NIEHS #05651.

1381 ACETAMINOPHEN PROTEIN ADDUCTS IN MOUSE LIVER AND SERUM AND HUMAN SERUM AFTER HEPATOTOXIC DOSES OF ACETAMINOPHEN DETECTED BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION.

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Acetaminophen-induced hepatotoxicity has been attributed to covalent binding of the reactive metabolite N-acetyl-*p*-benzoquinone imine to cysteine groups on proteins as an acetaminophen-cysteine conjugate. We report a high-performance liquid chromatography with electrochemical detection (HPLC-ECD) assay for the conjugate with increased sensitivity compared to previous methods. Previous methods to quantitate the protein-bound conjugate have used a competitive immunoassay, or radiolabeled acetaminophen. With HPLC-ECD, the protein samples are dialyzed then digested with protease. The acetaminophen-cysteine conjugate is then quantified by HPLC-ECD utilizing tyrosine as an internal reference. Acetaminophen protein adducts were detected in liver and serum as early as 15 min after hepatotoxic dosing of acetaminophen to mice. Adducts were also detected in the serum of acetaminophen overdose patients. Analysis of human serum samples for the acetaminophen-cysteine conjugate revealed a positive correlation between acetaminophen-cysteine conjugate concentration and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, and time to treatment with the antidote, N-acetylcysteine. Adducts were detected in the serum of patients even with relatively mild liver injury, as measured by AST and ALT. This assay may be useful in the diagnostic evaluation of patients with hepatotoxicity of an indeterminate etiology, for which acetaminophen toxicity is suspect.

1382 AN LC-ESI-MS/MS ASSAY FOR THE QUANTITATION OF CROTONALDEHYDE-DERIVED EXOCYCLIC 1, N^2-PROPANODEOXYGUANOSINE ADDUCTS.

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Crotonaldehyde is a genotoxic and carcinogenic α, β-unsaturated aldehyde. Endogenous formation of crotonaldehyde presumably occurs through lipid peroxidation. Crotonaldehyde is a proposed metabolite of butadiene and is also ubiquitous in our environment due to automobile exhaust and industrial processes. Upon reaction with DNA, crotonaldehyde forms, among several other classes of DNA adducts, a pair of diastereomeric exocyclic 1, N^2-propanodeoxyguanosine adducts. These propano adducts are present in two cell systems in which crotonaldehyde is mutagenic. The ubiquity and mutagenicity of crotonaldehyde point to a need for the development of biomarkers for the assessment of crotonaldehyde exposure and risk. A sensitive and specific adduct quantitation method employing mass spectrometry would permit measurements of adducts at low doses and comparisons between endogenous and exogenous adducts. To this end, we have initiated development of an LC-ESI-MS/MS assay to quantitate crotonaldehyde-derived exocyclic 1, N^2-propanodeoxyguanosine adducts (C-dGuo 1&2). Both analyte and [4, 5, 6, 8-$^{13}C_4$]-labeled internal standards for C-dGuo 1&2 have been synthesized and characterized by HPLC, UV, full-scan MS, and NMR. The first analyte standard to elute during HPLC has been analyzed by LC-ESI-MS/MS (SRM 338→222) to determine preliminary conditions for quantitation of these adducts and to determine their sensitivity to LC-ESI-MS/MS. LC-ESI-MS/MS has proven to be an extraordinarily sensitive means of detecting these adducts, as 750 amol (10^{-18}mol) injections of the analyte standard yielded signal to noise ratios of up to 114. Method development is continuing with more extensive LC-ESI-MS/MS work to optimize the detection of C-dGuo 1&2 in DNA samples, and to determine a cleanup procedure for enrichment of C-dGuo 1&2 in DNA samples. Following the completion of method development, we will quantitate C-dGuo 1&2 in a variety of DNA samples.

1383 EFFECT OF CYP2E1 GENOTYPE ON ACRYLONITRILE HEMOGLOBIN ADDUCTS.

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Acrylonitrile (ACN) is metabolized by two pathways: conjugation with glutathione, and oxidation to cyanoethylene oxide catalyzed by CYP2E1. Measurement of ACN metabolites indicated an absence of oxidation to cyanoethylene oxide in CYP2E1-null mice [Sumner et al., Chem. Res. Toxicol. 12: 1110-1116, (1999)]. Hemoglobin adducts provide a long-term measure of the internal dose of a reactive chemical. ACN reacts with hemoglobin to form a number of adducts, including *N*-(2-cyanoethyl)valine (CEVal). We have developed a new method for analysis of CEVal using LC-MS/MS. After a modified Edman degradation with phenylisothiocyanate as derivatizing agent, the resulting adducted valine phenylthiohydantoin derivative was isolated by solid phase extraction, and chromatographed by reverse phase HPLC. Elution was monitored by selected reaction monitoring on a PE Sciex API 3000 triple quadrupole mass spectrometer. The method can measure adducts from natural abundance and isotope labeled ACN. To investigate the impact of the CYP2E1 genotype on ACN internal dose, [1, 2, 3-^{13}C] ACN (20 mg/kg) was administered to wild type and CYP2E1-null mice, and blood was collected for CEVal analysis. In the wild type mice, CEVal levels were approximately 3 fold lower than in the CYP2E1-null mice (1487 ± 728 fmol/mg globin *vs.* 4863 ± 911 fmol/mg globin). These data indicate that, in the absence of CYP2E1 and the capability to oxidize ACN, the internal dose of acrylonitrile in the CYP2E1-null mice increased to greater than 3 times the levels in wild type mice.

1384 ESTIMATING POPULATION MEANS FOR THE DISTRIBUTIONS OF POLYCHLORINATED BIPHENYL CONGENERS IN CENSORED DATA SETS.

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Samples of milk from 99 lactating women were analyzed for 69 PCB congeners, DDE, Mirex, and hexachlorobenzene (HCB) (Kostyniak et al. Environmental Research 80:S166-174, 1999). Eight PCB congeners and one pesticide (DDE) were found in amounts above the limit of detection (LOD) in all 99 women. The remaining PCB congeners and pesticides were found in some, but not all, of the women. Values below the LOD are treated as censored data. Several methods have been suggested in the literature for estimating the mean and standard deviation of the population of concentrations for censored data sets. In the present study we examine the performance of five methods: substitution of the LOD or half the LOD (LOD/2), the maximum likelihood estimator (MLE), the restricted maximum likelihood estimator (RML) and a linear regression analysis (LR). We compare the estimators' performance in the eight PCBs and in DDE for which there is a complete data. The mean and standard deviation are calculated for each congener of the full data set. Censored data sets were created from the complete data set by dropping the smallest 10, 20, 30, 40, 50, 60, 70, 80, and 90 percent of the data. The methods were then compared for each degree of censoring, using the percent error as a measure of the bias of the estimates of the mean, and the ratio of the observed to estimated variance in the population as a measure of the relative efficiency of the estimator. The RML, MLE and LR gave estimates of the mean within 7% of the actual value for all levels of censoring. Although we could not statistically distinguish between the RML, MLE and LR, all three methods performed significantly better than substitution of the LOD or LOD/2. In conclusion the RML, MLE and LR are more useful than substitution of the LOD or the LOD/2 to estimate the population mean PCB congener concentration in censored data sets.

1385 SECULAR CHANGE OF BIOLOGICAL MONITORING MEASUREMENTS IN JAPAN.

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Partial amendments to the Japanese Ordinance on the Prevention of Lead Poisoning and the Japanese Ordinance of the Prevention of Organic Solvent Poisoning were made in 1989. As results, the measurement of lead in blood and urinary delta-aminolevulinic acid (ALA) became indispensable items of the occupational health examination for workers who handle lead. Also, the measurement of urinary metabolites of workers who handle eight kinds of organic solvents became mandatory. In order to support the national-wide biological monitoring program in Japan, seven major laboratories meet regularly to improve quality control and collect the measurement results after the enforcement of the amended ordinances. The annual number of measurements was about 100, 000 for lead in blood and ALA in urine, and about 500, 000 for the metabolites of the organic solvents, however, the total number of the subjects has slightly decreased for the last 10 years. The number of urine with high ALA concentration decreased drastically during the

10 years. The reasons seem to be not only the improvement of working environment but also the alteration of analytical methods from colormetric one to HPLC. The number of urine with high concentration of the metabolites also has decreased during the 10 years except hippuric acid. It can be concluded that occupational exposure to lead or the organic solvents has been decreased after the commencement of the national-wide biological monitoring.

1386 CHANGES OF URINARY AND BLOOD PORPHYRINS DURING PROLONGED EXPOSURE AND POST EXPOSURE TO PCBs, PB OR DIAZINON IN RATS.

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Porphyrins are intermediates in the biosynthesis of heme. The porphyrin pattern in excreta or tissues has been introduced as a useful biomarker for hazardous xenobiotics. In this study, we evaluated and compared the urinary and blood porphyrin profiles by treatment of PCBs (Aroclor1254), Pb or diazinon in rats. PCBs (10, 50, and 100 mg/kg bw), Pb(62.5, 250 and 1, 000 ppm) and diazinon(10, 30 and 90 mg/kg bw) were administered to SD male rats *via* gavage (PCBs and diazinon) or *via* drinking water (Pb) daily for 5 weeks. The highest dose of each chemical was selected as LD10. Uro, copro and protoporphyrin in urine and blood collected every week for 5 weeks of exposure and 5 weeks of post exposure were quantitated with fluorospectrometer. No clinical signs were observed by Pb and diazinon, while icterus and 15% lethality were induced by 100mg/kg PCBs. Three of urinary porphyrins and blood protoporphyrin were increased gradually and dose responsively in progress of PCBs exposure time and then urinary coproporphyrin came to peak at 5 weeks of exposure, urinary uroporphyrin and blood protoporphyrin at 2 weeks after withdrawal and urinary protoporphyrin at 3 weeks after withdrawal. They were continued to be higher than control until 5 weeks after PCBs withdrawal. By the treatment of Pb, the increase of urinary porphyrins and blood protoporphyrin was induced more quickly than PCBs. That is, urinary porphyrins were increased rapidly, came to peak at 2 weeks of exposure and returned to normal levels as soon as withdrawal and blood protoporphyrin began to increase from 1 week, peaked at 5 weeks of treatment and continued to be higher than control during withdrawal period. In diazinon case, urinary and blood porphyrins were not so much sensitively affected as PCBs and Pb, just showing weak increase of urinary coproporphyrin. These results demonstrate that urinary and blood porphyrin profiles confer characteristic biomarkers and useful tools for exposure assessment of PCBs, Pb and diazinon.

1387 PLATINUM LEVELS IN NASAL LAVAGE ARE NOT RELATED TO TRAFFIC DENSITY BUT TO NASAL INFLAMMATION.

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Platinum (Pt) is a well-known constituent of particles emitted by autocatalysts. To evaluate Pt as a potential marker for traffic related particle exposure, we investigated Pt content along with non-traffic related metals (Cr, V) in coarse and fine particulate matter (PM) sampled in 4 areas with different traffic density as well as in nasal lavage fluid (NALF) of 63 children living in these areas. Pt, Cr and V content of coarse and fine PM were not significantly different between different sites, although CO emmission as a validated traffic marker during the sampling interval was clearly different (ANOVA, $P < 0.05$). In addition, concentrations of these metals in nasal lavage of children living in these locations were not significantly different. However, a significant correlation between Pt levels in NALF and total number of cells or interleukin-8 as markers for inflammatory status of the nose was found. No relationship was present between inflammation and Cr and V levels in nasal lavage.We suggest that Pt is a marker for traffic-related PM that are able to induce inflammation in the upper respiratory tract.

1388 USE OF DNA ARRAYS TO MONITOR GENE EXPRESSION IN BLOOD AND UTERUS FROM LONG-EVANS RATS FOLLOWING 17-BETA-ESTRADIOL EXPOSURE - A NEW APPROACH TO BIOMONITORING ENDOCRINE DISRUPTING CHEMICALS USING SURROGATE TISSUES.

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Evidence exists that environmental xenobiotics are disrupting the endocrine systems of certain wildlife species, resulting in developmental abnormalities and reproductive impairment. Although the relationship between human disease and exposure to such endocrine disrupting chemicals (EDCs) is unknown, it is believed that EDCs present a potential threat to public health. Gene expression analysis offers the ability to identify preclinical effects of exposure to EDCs and provide mechanistic data useful for risk assessments, and could ultimately provide a vehicle for developing early diagnostic and preventative measures in at-risk populations or individuals. Since apparently healthy human reproductive organs cannot be sampled directly, a method to utilize more accessible tissues must be developed. We propose that gene expression changes in accessible tissues such as blood will often reflect those in inaccessible tissues, thus offering a convenient biomonitoring method for providing insight into the effects of environmental toxicants on target tissues. In this pilot study, gene expression changes in peripheral blood lymphocytes (PBLs) were compared with those in the uteri of adult rats to identify genes that were altered in both tissues following estradiol treatment. Ovariectomized rats were treated with either 17-beta-estradiol or vector control for three days. Uteri and PBLs were harvested, and total RNA extracted and hybridized to Clontech Atlas rat 1.2i arrays. A number of genes were found to demonstrate a similar degree of expression change that was treatment-, but not tissue-specific, demonstrating that accessible tissues such as PBLs can act as surrogate tissues for observing gene expression changes in inaccessible target tissues.

1389 PLATELET MAO-B ACTIVITY AS A STATE MARKER OF ALCOHOLISM.

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This study evaluated time-related changes in platelet monoamine oxidase B (MAO) activity in a homogeneous Italian cohort of alcoholic-dependent subjects (n = 98) during early abstinence and the effect of potential confounding factors, such as gender and smoking status, on the temporal trend of this enzyme activity. While still under the influence of ethanol (time point T1) mean MAO activity in alcoholics was 6.4 ± 3.1 nmol/mg protein/hr. This returned within the control range (9.9 ± 0.9 nmol/mg protein/hr) after 8 days of withdrawal (T2), and remained stable thereafter (T3 and T4: 15 and 22 days of abstinence, respectively). Alcohol intake cessation was confirmed by serum %carbohydrate-deficient transferrin (CDT), which was pathologically above the reference limits (6%) at T1 (7.8 ± 3.3%), decreased to 6.6 ± 2.1% at T2 and reached physiological values at T3 and T4. In cirrhotic alcoholics %CDT did not decrease over time, while MAO activity was comparable to that of abstinent noncirrhotic alcoholics. The mean values of other liver function tests were elevated at the first evaluation (GGT, serum gamma-glutamyltransferase: 641 ± 894 U/l; AST, aspartate aminotransferase: 119 ± 70 U/l; ALT, alanine aminotransferase: 86 ± 56 U/l), decreased during hospitalization but remained above the reference values at the patient's dismissal (GGT: 231 ± 487 U/l; AST: 39 ± 34 U/l; ALT: 47 ± 37 U/l). MCV (mean corpuscular volume) values were elevated at admission and remained so during hospitalization. Neither gender nor tobacco smoking affected the temporal pattern of MAO activity during early withdrawal. MAO-B can be regarded as a state-marker of alcohol consumption. The temporal pattern of its activity may be used for the diagnostic assessment of alcoholism and early abstinence, regardless of gender and smoking status. (Supported by grant QLRT-2001-00186 from the European Commission).

1390 CYTOCHROME OXIDASE ACTIVITY AND CHOLINERGIC MUSCARINIC RECEPTOR BINDING AS PERIPHERAL MARKERS OF CARBON MONOXIDE NEUROTOXICITY.

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Carbon monoxide (CO), a widely spread neurotoxicant, has been shown to alter cytochrome oxidase C (COX) activity and cholinergic parameters in the brain of acutely exposed rodents. A decrease in COX activity has also been observed in the lymphocytes of acutely poisoned humans. In this study we investigated the early and delayed effects of *in vivo* CO exposure on cholinergic muscarinic receptor (MR) binding and COX activity in the rat brain and whether CO-induced central changes would be reflected by similar alterations in peripheral lymphocytes. Male Wistar rats were exposed by inhalation to 500 ppm CO for 6 hr/day, 5 days/wk for 4 wks. One and 7 days after the end of the treatment, no changes were detected in 3H-QNB binding to MR in the cerebral cortex, cerebellum and hippocampus (control values, mean ± SD, were for cerebral cortex, cerebellum and hippocampus 171 ± 45, 77 ± 7 and 245 ± 53 fmol/mg protein, respectively) as well as in the lymphocytes (24 ± 19 fmol/million cells). Similarly ineffective was repeated CO exposure towards brain (124 ± 25 nmol/min/mg protein) and lymphocyte (26 ± 9 nmol/min/mg protein) COX activity at the same time points. Acute CO inhalation (2400 ppm, 1 hr) did not modify central and peripheral COX activity and MR

binding 1 hr, 1 day, 3 and 7 days after treatment. Studies on other possible CO targets (such as cGMP) are in progress in order to identify valuable peripheral biomarkers of central effect (Supported by grant QLK4-CT99-01356 from the European Commission).

1391 SENSITIVE EARLY INDICATORS OF HEPATIC AND KIDNEY DAMAGE IN WORKERS EXPOSED TO JET FUEL(JP-8).

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Jet Propellant type 8 (JP-8)is used for all US military operations. Because of the widespread exposure and concern for potential health effects, three hundred twenty-four US Air Force (USAF) active duty personnel participated in a study at six USAF bases between April and September, 2000. Animal & human studies suggest that exposure to fuels and solvents can adversely effect the liver and kidney. Glutathione transferase (GST) isoenzymes possess specific patterns of distribution in organs and are released to biological fluids in response to toxic insult. ELISA techniques can be used to measure those GSTs associated with liver and kidney damage. Pre- and post-shift serum and urine samples were collected from USAF personnel and commercial GST assays were used to measure alpha GST in serum (marker for liver damage) and alpha and pi GST in urine which are associated with proximal and distal tubule damage, respectively. Exposed workers were tank-entry personnel with at least nine months of persistent exposure to jet fuel; the unexposed group were USAF personnel with no significant exposure to fuels or solvents. Levels of serum hepatic alpha-GST, and urinary nephritic alpha- and pi-GST in the study subjects fell within the normal range for healthy subjects. No differences were observed indicative of liver or kidney damage attributable to any of the exposure, lifestyle and other demographic variables examined. Creatinine, used to normalize urinary GSTs was elevated in post-shift samples from the highest exposure category. This study group represents a very healthy segment of the population. Sensitive measures for liver and kidney damage did not detect any adverse effects in this study group. Evidence of elevated creatinine in the mean post-shift samples of the high exposure category was seen. However, while these values are within normal clinical ranges, they are consistent with concentrated urine indicative of mild dehydration.

1392 A BIOLOGICAL LIMIT VALUE (BLV) FOR OCCUPATIONAL EXPOSURE TO PERFLUOROOCTANOATE.

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A biological limit value (BLV) of 5 ppm for perfluorooctanoate (PFOA) in the serum of workers has been established by 3M. Ammonium PFOA (APFO) is a polymerization aid used in fluoropolymer production. The 3M BLV represents the best scientifically-based estimate of a concentration of PFOA in a biological fluid (serum) that, if present, even on a chronic basis, is not expected to correlate with a risk of adverse health effects in workers. Serum levels above the 5 ppm BLV do not necessarily imply a health risk. A significant body of toxicological, epidemiological, and medical surveillance information is available for PFOA and APFO to support a BLV of 5 ppm PFOA in serum. The results of toxicology studies indicate that the major target organ of APFO is the liver. Oral administration of APFO to monkeys at 3 and 10 mg/kg/d for six months resulted in mean serum concentrations of approximately 50 and 70 ppm, respectively, and was associated with increases in both absolute liver weight and liver-to-body weight ratios without gross or histopathological abnormalities or changes in clinical chemistries. Approximately 90 percent of 3M workers exposed to APFO have had serum PFOA levels below 10 ppm. Investigators have not observed any exposure-related clinical hepatic toxicity or hormonal changes in these workers. The repeated analyses, to date, provide reasonable assurance that workers with serum PFOA levels below 10 ppm do not experience exposure-related clinical hepatotoxicity; however, there have been too few employees with serum PFOA concentrations above 10 ppm to draw statistically-significant conclusions regarding a lack of clinical hepatotoxic effects with serum PFOA levels at or above 10 ppm.

1393 DIFFERENT RESPONSES IN THE PATTERN OF EXPRESSION OF P53 CHALLENGED WITH ARSENIC.

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Sponsor: M. Cebrián.

Different pattern of expression of the p53 protein were observed when lymphocyte cultures were challenged with arsenic. To evaluate the reproducibility of this effect we determined the presence of p53 in blood samples from 22 healthy individuals, using 4 different treatment conditions: (1) non-stimulated lymphocytes (Go); (2) lymphocytes stimulated with phytohemagglutinin during 48h (Control); (3) stimulated lymphocytes treated with arsenic(As); or (4) with actinomycin-D (AcD) during the last 24h. Proteins were obtained at the end of culture, and measured by immunoblot. Data show three different patterns of p53 expression : I) 16 individuals had bands in As and AcD treated cultures but not in Go; II) 4 individuals in addition to the previous pattern, showed a band in Go; III) 2 donors did not show any p53 response. No relation of these expression-pattern differences with cell proliferation could be established. Since we have found a higher frequency of polymorphisms in the intron 1 (Bgl II P53) among breast cancer patients, we analyzed the p53 expression in females with and without polymorphism. No relationship among both parameters was found. The expression of p53 in lymphocyte cultures could be of relevance in the understanding of individual susceptibility to xenobiotics and might have a great potential as a biomarker.

1394 BIOMARKER CHARACTERIZATION OF ASPHALT FUME EXPOSURE.

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Each year more than 60 million tones of asphalt are produced and used worldwide. It has been estimated that approximately two million workers were employed and potentially exposed to asphalt. Health concern from exposure to asphalt is related to its content of hazardous chemicals, such as polycyclic aromatic hydrocarbons (PAHs). The development of a biomarker to provide an assessment of the integrated external exposure following uptake by living system through inhalation is valuable for this kind of complex mixture exposure. Many PAHs are metabolized to hydroxyl-PAHs and excreted in urine; therefore, urinary hydroxyl-PAH was used as a biomarker for asphalt fume exposure in this study. Female Sprague-Dawley rats and B6C3F1 mice were exposed to asphalt fume in a whole body inhalation chamber for 5 or 10 days (3.5 hrs/day, 71-97 mg/m ^{3}). Clean air chamber animals were used as controls. The test asphalt was the type used by paving industry throughout the Midwestern United States. The fume was generated under simulated road paving conditions. Urine sample preparation for the analysis of hydroxyl-PAHs metabolites included enzymatic digestion, solid phase extraction, and isotope dilution GC/MS and microflow LC-Q-TOF MS detections. Four priorities PAH metabolites, including, 1-hydroxypyrene, 9-phenanthrenol, 1-chrysenol, and 2-naphthalenol were identified and determined in urine. Isotope-dilution has proved to be useful for quantification of those metabolites. Preliminary results indicated that urinary PAH metabolite could be a sensitive biomarker following asphalt fume exposure. The method developed in this study has potential application to monitor of PAHs from occupational mixture exposure.

1395 BIOMARKERS OF OXIDATIVE STRESS STUDY: ARE OXIDATION PRODUCTS OF LIPIDS MARKERS IN CCL4 POISONING?

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Oxidation products of lipids in plasma and urine of rats were measured as part of a comprehensive, multilaboratory validation study searching for non-invasive biomarkers of oxidative stress. This is the second report of the nationwide Biomarkers of Oxidative Stress Study (BOSS). The time (2, 7, and 16 h) and dose (120 and 1200 mg/kg ip)-dependent effects of CCl4 on concentrations of lipid hydroperoxides, TBARS, malondialdehyde (MDA) and isoprostanes were investigated with different techniques. Plasma concentrations of MDA and isoprostanes (measured by GC/MS) and urinary concentrations of isoprostanes (measured with an immunoassay) were increased in CCl4 treated rats in a time- and dose-dependent manner. All other products were not changed by CCl4 or showed only high-dose and/or single time point effects. It is concluded that measurements of MDA and isoprostanes concentrations in plasma and urinary isoprostanes are promising candidates for general biomarkers of oxidative stress. Acknowledgements: B Ames [1], N Brot [2], G Fitzgerald [3], R Floyd [4], M George [5], G Hatch [6], J Heineke [7], K Hensley [4], J Lawson [3], L Marnett [8], J Morrow [9], D Murray [10], J Plastaras [8], L J Roberts [9], M Shigenaga [1], R Sohal [11], Jie Sun [10], R Tice [12], D H Van Thiel [5], D Wellner [13], P Walter [1]. [1] Children's Hospital Oakland Res. Inst.., Oakland, CA., [2] Hospital for Special Surgery and Department. Microbiol. Immunol., Weill Med.. College of Cornell University., NY, NY., [3] Center for Exp. Therapeutics., University. Pennsylvania, Philadelphia, PA., [4] OMRF, Oklahoma City, OK., [5] Loyola University. Med.. Center, Maywood, IL., [6] USEPA, Research Triangle Park, NC., [7] Department. Mol. Biol. Pharmacology, Washington University. School of Medicine, St. Louis, MI., [8] Department. Biochem, School of Medicine, Vanderbilt University., Nashville, TN., [9] Department. Pharmacol and Medicine, Vanderbilt University., Nashville, TN., [10] Oxis Inc.., Portland, OR., [11] Department. Biol.

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1396 URINARY BISPHENOL A GLUCURONIDE AS A BIOMARKER FOR BISPHENOL A EXPOSURE.

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The estrogenic effects of bisphenol A (BPA) have been reported in E-screen assay and in *vivo* studies of rodents, however, the exposure levels and health effects of BPA in humans are still unknown. We assessed the BPA exposure by the measurement of urinary BPA-glucuronide. University students and workers who handled BPA diglycidyl ether (DGEBPA) were evaluated in the present study. There urinary BPA levels were measured using a HPLC with fluorescence detector. BPA-glucuronide concentration was calculated by the subtraction of BPA measurement without β-glucuronidase treatment from BPA measurement with β-glucuronidase treatment. The method developed for urinary BPA determination shows a linear calibration, and good recovery rates and reproducibility. The urinary BPA levels of the students in 1992 were significantly higher than those of 1999, and BPA levels in the students who habitually drink more coffee and tea tend to be higher than those of students who drink less. The urinary BPA levels of BPADGE-workers were significantly higher than those of the control workers. It is thought that the high levels of urinary BPA of the students in 1992 were caused by drinking BPA dissolved from inside-wall coating of coffee and tea cans. However, our recent BPA exposure seems to be very low because the inside coatings had been changed. Although the workers who handle BPADGE have a risk of BPA exposure, their exposure levels were almost same as those of the university students in 1992.

1397 RAPID GENOTYPE METHODS TO DISTINGUISH SINGLE NUCLEOTIDE POLYMORPHISMS WITHIN THE HUMAN NAT1 AND NAT2 GENES.

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N-acetyltransferase 1 (NAT1) and 2 (NAT2) catalyze the activation and deactivation of aromatic amine drugs and carcinogens. Genetic polymorphisms in NAT1 and NAT2 have been associated with the incidence of cancers related to exposures to aromatic and heterocyclic amines found in cooked foods, cigarette smoke, and the environment. We designed facile *NAT1* and *NAT2* genotyping methods that distinguish among the most frequent and/or functional nucleotide polymorphisms in the *NAT1* and *NAT2* coding regions and *NAT1* 3*acute*-untranslated region (UTR). The single nucleotide polymorphisms detected in NAT1 are: C97T (R33Stop), C190T (R64W), G445A (V149I), C559T (R187Stop), G560A (R187Q), A752T (D251V), T1088A (3*acute-*UTR), C1095A (3*acute*-UTR). All NAT1 alleles (*NAT1*3, NAT1*4, NAT1*10, NAT1*11A, NAT1*11B, NAT1*14A, NAT1*14B, NAT1*15, NAT1*17, NAT1*19, NAT1*22*) except very rare ones are detected by this method. The single nucleotide polymorphisms detected in the *NAT2* genotyping method are: G191A (R64Q), C282T (silent), T341C (I114T), C481T (silent), G590A (R197Q), A803G (K268R), G857A (G286T). This method can detect all except very rare human *NAT2* alleles including *NAT2*4, NAT2*5A, NAT2*5B, NAT2*5C, NAT2*5D, NAT2*5E, NAT2*6A, NAT2*6B, NAT2*6C, NAT2*7A, NAT2*7B, NAT2*11, NAT2*12A, NAT2*12B, NAT2*12C, NAT2*13, NAT2*14A, NAT2*14B, NAT2*14C, NAT2*14D, NAT2*14E, NAT2*14F and NAT2*14G.* These genotyping methods do not require post-PCR processing or the use of radioactivity, and therefore are very suitable for high-throughput applications. Partially support by USPHS grant CA-34627 from the National Cancer Institute

1398 COMPARING THE EFFECTS OF ALTEPLASE (TPA), TENECTEPLASE (TNK-TPA) AND RETEPLASE (RPA) ON HEMOSTATIC PARAMETERS IN A RABBIT MODEL OF PROLONGED INFUSION.

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Urokinase unavailability has led clinicians to use other thrombolytics for catheter-directed thrombolysis (CDT) in treating acute limb ischemia and deep vein thrombosis. Despite the widespread clinical use of thrombolytics, there is no preclinical safety data evaluating the effects of these agents on coagulation parameters when administered using CDT. This study evaluated the effect of tPA, TNK-tPA, or rPA on hemostatic parameters using a rabbit model and infusion techniques similar to clinical practice. tPA, TNK-tPA, or rPA were infused (IV) at doses corresponding to 0, 0.5, 1.0, 2.0, and 10 (mg/hr, t-PA, TNK; U/hr, r-PA) in a 70 kg human. Arterial blood samples were collected at 0, 0.5, 1, 2, 3, and 4 hours for coagulation parameters (PT, aPTT, fibrinogen, plasminogen and alpha2-antiplasmin). A dose related change in PT and systemic fibrinogen was exhibited with all thrombolytics; however, rPA had a greater increase in PT and greater depletion of systemic fibrinogen than tPA or TNK-tPA. tPA exhibited reduced alpha2-antiplasmin depletion (>65% of baseline at all doses) compared to TNK-tPA (20% of baseline with 0.143 mg/kg TNK-tPA) and rPA (0, 40 and 50% of baseline with 0.143, 0.029 and 0.014 U/kg rPA, respectively). Plasminogen levels exhibited similar dose related changes. This data indicates significantly less alteration of systemic hemostasis parameters using highly fibrin-specific agents, tPA and TNK-tPA, compared to rPA. Consequently, tPA and TNK-tPA may provide a better safety profile with less systemic fibrinolysis when used for peripheral vascular applications; further comparative studies are warranted.

1399 EARLY EFFECT OF PHOSPHODIESTERASE (PDE) IV INHIBITION ON LEUKOCYTE-ENDOTHELIAL CELL INTERACTION: FUNCTIONAL RELEVANCE OF ENHANCED EXPRESSION OF INTEGRINS AND ADHESION MOLECULES TO EXPERIMENTAL VASCULITIS IN RATS.

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PDE inhibitors have been shown to cause vasculitis in nonclinical toxicology studies, typically in rats. Vasculitis, such as that induced by the type IV PDE inhibitor CI-1018 is characterized by medial necrosis with perivascular mixed inflammatory cell infiltrates. Underlying mechanisms associated with initiation of the vascular inflammatory process are poorly understood. In the present studies we investigated the ability of CI-1018 to modulate early events of leukocyte-endothelial cell interactions, leukocyte surface expression of betta2-integrins, and gene regulation of the corresponding endothelial counter ligand intercellular adhesion molecule-1 (ICAM-1). Leukocyte adhesion and rolling activity were studied in female Wistar rats by intravital microscopy (IVM) between 1.5 and 4 hours following a single oral dose of CI-1018 at 750 mg/kg. IVM revealed an increase in the number of adherent and rolling leukocytes in mesenteric postcapillary venules. In a separate experiment, flow cytometric analysis showed that neutrophil surface integrin expression was upregulated 4 hours after a single dose (CD11a and CD11b) and 72 hours after 3 daily doses (CD11a, CD11b and CD18) of CI-1018, respectively. RT-PCR analyses revealed a rapid upregulation of ICAM-1 mRNA that occurred within 4 hours of a single dose with CI-1018. Using an *in vitro* leukocyte-endothelial adhesion assay, significantly more neutrophils from CI-1018-treated rats adhered to TNF-alpha-activated cultures of rat endothelial cells compared to neutrophils from controls. Blockade with anti-CD18 antibody significantly decreased neutrophil adhesion to the endothelial cell monolayer. These results suggest that CI-1018 induces early leukocyte adhesion which is mediated, at least in part, by betta2-integrins and the endothelial ligand ICAM-1. These events likely have functional relevance to the pathogenesis of PDE IV inhibitor-induced vascular inflammation in rats.

1400 EXPRESSION OF ENDOTHELIAL AND INDUCIBLE NITRIC OXIDE SYNTHASE AND FORMATION OF PEROXYNITRITE-MODIFIED PROTEINS IN PHOSPHODIESTERASE (PDE) INHIBITOR-INDUCED VASCULITIS IN RATS.

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The type IV PDE inhibitor CI-1018 has been shown to cause vascular lesions in nonclinical toxicology studies, typically in rats. It has been proposed that excessive release of nitric oxide by inducible nitric oxide synthase (iNOS) and subsequent formation of highly reactive peroxynitrite plays a role in tissue damage in PDE inhibitor-induced vascular injury. This study was conducted to determine the putative cellular source of iNOS, to identify the distribution of nitrotyrosine residues as a measure of peroxynitrite production, and to evaluate their association with vascular injury induced by CI-1018. Four groups of female Wistar rats were given doses of vehicle or CI-1018 at 750 mg/kg daily by gavage for up to 3 days. One group of CI-1018-treated rats was sacrificed at 24 hours after each dose. Endothelial nitric

oxide synthase (eNOS), iNOS and nitrotyrosine were evaluated by immunohistochemistry in paraffin sections of mesenteric artery. Arteries showed intense iNOS immunostaining of endothelial cells in rats that received the full course of 3 daily doses of CI-1018 and only in sections that showed mild to marked vascular injury. eNOS was evident in endothelial cells without differences in staining intensity between control and CI-1018-treated groups. Nitrotyrosine-positive cells were detected only on reactive oxygen species-producing inflammatory cells, distinct from iNOS-producing endothelial cells. Nitrotyrosine-postitive staining was present exclusively in inflammatory cells at 72 hours, coinciding with the peak in iNOS expression in mesenteric artery. It is concluded that PDE IV inhibitor-induced vascular injury in rats is associated temporally with the induction of iNOS in endothelial cells and with production of peroxynitrite by inflammatory cell infiltrates, but direct evidence of peroxynitrite-mediated vascular injury remains to be demonstrated.

1401 EFFECTS OF VARIOUS COMPOUNDS ON ACTION POTENTIAL PARAMETERS IN ISOLATED DOG PURKINJE FIBERS.

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The QT interval on the electrocardiogram is the time from the onset of ventricular depolarization (the Q wave) to completion of repolarization (the end of the T wave) in the axis of the lead chosen for measurement. The QT interval is of special interest in view of potential therapeutic agents that can influence the duration of the interval and the known association between QT prolongation and the potential for malignant ventricular arrhythmias. As part of preclinical safety assessment packages, both *in vitro* and *in vivo* systems can be used to demonstrate effects of chemicals on electrophysiological and electrocardiographic parameters. *in vitro* Purkinje fiber preparations from a variety of laboratory species are considered to be suitable experimental models provided that the major ionic currents underlying the investigated cardiac action potential do not differ substantially from those encountered in human cardiac tissues. In this study, the effects of various compounds on action potential parameters were determined in isolated canine cardiac Purkinje fibers using intracellular microelectrodes. Action potential parameters measured were disatolic membrane potential, maximum rate of depolarization of action potential upstroke, overshoot action potential, action potential amplitude and action potential duration at 30, 50 and 90% repolarization. Reference standards of dl-sotalol and l-phenylephrine were observed to prolong action potential duration, while lidocaine was found to shorten action potential duration. Lidocaine shortened action potential duration at all concentrations and frequencies tested. Interestingly, the effects of the l-phenylephrine standard on action potentials were found to be time-dependent and biphasic. Low concentrations of phenylephrine rapidly prolonged action potential duration, but the maximum extent of this prolongation was not maintained throughout the 30 min exposure period. The standard dl-sotalol was observed to prolong action potential duration in a

1402 PRE-CLINICAL ASSESSMENT OF CARDIOTOXICITY AND IN COMMON MARMOSETS.

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Appropriate species selection for safety pharmacology and drug toxicity testing is essential for the development of novel pharmaceuticals for human use. The purpose of this study was to obtain information on both the cardiovascular system in conscious common marmosets using a telemetry system, and the characteristics of marmoset ventricular myocytes through measurement by the patch-clamp method. experiment: Male and female marmosets (1-3 years old) were used. The heart was removed and perfused with Ca -free Tyrode solution containing collagenase through the coronary artery, using a Langendorff perfusion system. The ventricles were cut into pieces and dispersed with a pipette in order to isolate ventricular myocytes. Action potential (AP) and ion current of ventricular myocytes were measured by the patch-clamp method. experiment: Male marmosets (1-3 years old) were used. In conscious, unrestrained marmosets, ECG (similar to CM-V5 in Holter recording), heart rate and blood pressure were continually measured by a telemetry system. In the experiment, astemizole prolonged APD (AP duration at 90% repolarization) of ventricular myocytes. E4031, an inhibitor of I (rapid phase of K current in AP) also prolonged APD90. In the experiment, astemizole at 30 mg/kg (p.o.) or 3 mg/kg (i.v.) was shown to induce QT interval prolongation, resulting in torsades de pointes-like ventricular tachycardia. In conclusion, the assessment demonstrated the scientific value of the marmoset as a predictor of adverse effects in humans.

1403 INDUCTION OF ANTIOXIDANTS BY 3H-1, 2-DITHIOLE-3-THIONE IN CARDIOVASCULAR CELLS AFFORDS PROTECTION AGAINST OXIDATIVE CELL INJURY.

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Considerable evidence suggests that reactive oxygen species (ROS) are critically involved in the pathogenesis of cardiovascular diseases, particularly atherosclerosis. Consistent with this concept, consumption of antioxidants, such as vitamin E, has been shown to be causally related to a decreased incidence of atherosclerosis. However, whether induction of endogenous cellular antioxidants by chemicals (drugs) also affords protection against oxidative cardiovascular injury has not been extensively investigated. In this study, using rat aortic A10 cells as an *in vitro* system we have characterized the induction of cellular antioxidants by the antioxidant-inducing agent, 3H-1, 2-dithiole-3-thione (D3T) and the protective effects against ROS-mediated injury in cardiovascular cells. Incubation of A10 cells with D3T for 24 hours resulted in a significant dose-dependent induction of a battery of cellular antioxidants, including reduced glutathione (GSH), GSH peroxidase, glutathione reductase, catalase, and superoxide dismutase. To further examine the protective effects of the induced endogenous antioxidants against oxidative cell injury, A10 cells were pretreated with D3T and then exposed to xanthine oxidase (XO) plus xanthine, a system that produces superoxide and hydrogen peroxide. We observed that D3T pretreatment of A10 cells led to significant protection against XO/xanthine-induced cytotoxicity as determined by 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) reduction and morphological changes. Taken together, this study demonstrates that a number of endogenous antioxidants in vascular smooth muscle cells can be induced by exposure to D3T, and that this chemical induction of cellular antioxidants is accompanied by markedly increased resistance to ROS-mediated cardiovascular cell injury.

1404 CHARACTERIZATION OF CELLULAR GLUTATHIONE AND GLUTATHIONE S-TRANSFERASE, AND THEIR INDUCIBILITY BY 3H-1, 2-DITHIOLE-3-THIONE IN RAT AORTIC SMOOTH MUSCLE A10 CELLS: PROTECTION AGAINST REACTIVE ALDEHYDE-INDUCED TOXICITY.

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There is increasing evidence that aldehydes generated endogenously during the degradation process of biological molecules are involved in the pathogenesis of cardiovascular diseases, such as atherosclerosis. Because glutathione (GSH) and GSH S-transferase (GST) are a major cellular defense against the toxic effects of reactive aldehydes, in this study we have characterized the inducibility of GSH and GST by 3H-1, 2-dithiole-3-thione (D3T) and their protective effects against acrolein and 4-hydroxy-2-nonenal (4-HNE)-induced toxicity in rat aortic smooth muscle A10 cells. Incubation of A10 cells with micromolar concentrations of D3T resulted in a concentration- and time-dependent induction of both GSH and GST. Furthermore, the levels of GSH and GST remained higher than basal levels 72 hours after removal of D3T from the culture media. To examine the protective effects of D3T-induced GSH and GST against reactive aldehyde-mediated toxicity, A10 cells were pretreated with D3T and then exposed to acrolein or 4-HNE. Pretreatment of A10 cells with D3T resulted in a marked decrease of acrolein or 4-HNE-induced toxicity as determined by MTT reduction assay and morphological changes. To further demonstrate the involvement of GSH and GST in protecting against acrolein and 4-HNE-induced toxicity, buthionine sulfoximine (BSO) and sulfasalazine were used to inhibit cellular GSH biosynthesis and GST activity, respectively. Either depletion of cellular GSH by BSO or inhibition of cellular GST by sulfasalazine led to a marked potentiation of acrolein or 4-HNE-induced toxicity in A10 cells. Taken together, our results demonstrate that both GSH and GST in aortic smooth muscle cells can be induced by D3T, and that this increased cellular defense affords protection against reactive aldehyde-induced cardiovascular cell injury.

1405 THE FORMATION OF PEROXYNITRITE AND INDUCTION OF THE INFLAMMATORY MEDIATOR, CYCLOOXYGENASE-2, ARE IMPORTANT FACTORS IN ARSENIC-RELATED CARDIOVASCULAR DISEASE.

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There is substantial epidemiological evidence linking arsenic-contaminated drinking water with an increased risk of cardiovascular disease. This includes decreased cardiac function as well as peripheral vascular diseases and atherosclerosis. The

mechanisms behind these phenomena are largely unknown. The purpose of this study was to determine whether arsenic could contribute to the chronic inflammatory and oxidative stress symptoms characteristic of atherosclerosis. In endothelial cells treated with sodium arsenite, we saw a significant increase in peroxynitrite, an oxidant formed from nitric oxide and superoxide that is also capable of nitrating protein tyrosine residues. This follows the pathology seen in atherosclerotic plaques in which the observed increase in nitrotyrosine residues is believed to be due to the presence of peroxynitrite. Peroxynitrite formation was confirmed by western blotting of a COX-2 immunoprecipitation probed with a nitrotyrosine antibody. There was increased nitration of the COX-2 protein in arsenic treated cells compared to controls. The COX-2 protein itself was also up-regulated in response to arsenic exposure, indicating an increase in the inflammatory response in endothelial cells. This was supported by an increase in the synthesis of PGE2 following exposure to arsenic. The results of this study suggest an increase in reactive species, particularly peroxynitrite, in endothelial cells exposed to arsenic. Furthermore, induction of important inflammatory mediators such as the COX-2 enzyme may exacerbate the inflammatory state typical of atherosclerosis.

1406 METALLOTHIONEIN PROTECTION AGAINST CADMIUM INDUCED CARDIOTOXICITY IN MICE.

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Cadmium (Cd) is a heavy metal environmental pollutant linked to the development of cardiovascular diseases. In murine models of Cd exposure, the effects of Cd are manifested by significant increase in blood pressure as well as heart morphological alterations. The sensitivity of the heart to Cd could be due to the low level of a thiol-rich, metal-binding protein, metallothionein (MT), in the tissue. The present study was thus undertaken to test the hypothesis that elevation of MT in the heart makes this organ resistant to Cd toxicity. A cardiac-specific MT-overexpressing transgenic (MT-TG) mouse model was used and cardiotoxicity was analyzed by evaluating performance of left ventricle and heart morphological changes. MT-TG mice and non-TG controls were treated with $CdCl_2$ (6 mg/kg) for 48 hrs. This treatment lead to a significant decrease in heart rate, maximum left ventricle pressure, and the first derivative of intraventricular pressure (dP/dt). On the other hand the duration of contraction and relaxation and the time constant (Tau) were prolonged. These changes were more pronounced in non-TG than in MT-TG animals. In addition, an increase in contractility caused by isoproterenol (Iso) administration in Cd-treated non-TG animals was more pronounced in comparison to saline treated animals; whereas, Cd-treated MT-TG animals responded in a similar manner to control. The direct effect of MT on Cd toxicity in cardiomyocytes, the contractile units of the heart was determined using primary neonatal mouse cardiomyocyte cultures. An MTT assay was performed for the assessment of cell viability; treatment with 0.1 μM Cd for 1 hr lead to a 65% decrease in non-TG cells whereas this dose lead to only a 50% decrease in viability in MT-TG cardiomyocytes. Results of this study demonstrate that elevation of MT in transgenic animals protects against Cd-induced cardiotoxicity manifested by functional alterations; furthermore cardiotoxicity most likely occurred in cardiomyocytes as demonstrated by the results obtained from cell cultures.

1407 MYOCARDIAL PROTECTION BY METALLOTHIONEIN FROM ISCHEMIA/REPERFUSION INJURY.

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Ischemia-reperfusion causes depressed myocardial function and associated deleterious morphological alterations that lead to heart failure. Metallothionein (MT) has been shown to provide cardioprotection against a variety of insults. This study was undertaken to test the hypothesis that MT also protects the heart form injury induced by ischemia/reperfusion. An open-chest coronary artery occlusion and reperfusion procedure to produce ischemia/reperfusion-induced left ventricle infarction was used. A total of 40 male MT-overexpressing transgenic and wild type mice were divided into 4 groups. The mice were subjected to 30 min of anterior descending coronary occlusion followed by a reperfusion for 24 hours. A sham surgery was applied to control groups. The MT concentrations in transgenic mouse hearts were ~10 fold higher than that in wild type controls. Myocardial infarction was significantly inhibited in the MT transgenic mice. The infarct sizes averaged 35% of the region at risk in transgenic and 61% in wild mice respectively. This inhibition correlated with the suppressed cardiac morphological and functional alterations, as determined by Hematoxylin-Eosin staining and heart function performance. In additon, ischemia-reperfusion-induced reactive oxygen species generation and lipid peroxidation were significantly inhibited in the transgenic myocardium. These results thus demonstrate that MT plays an important role in protection of the heart from ischemic/reperfusion injury. This cardioprotective effect of MT correlates with its antioxidant action. (Supported in part by NIH grants HL63760 and HL 59225)

1408 THE CORRECTION OF QT INTERVAL IN CONSCIOUS AND ANAESTHETISED BEAGLE DOGS USING DIFFERENT FORMULAE.

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During the last decade some non-cardiovascular drugs already on the market were revised for clinical use on the basis of possible prolongation of QT interval, a pro-arrhythmic event which can degenerate into a fatal ventricular arrhythmia (torsade de pointes). Therefore, starting from 1997, QT assessment for human medicinal products has attracted the attention of regulatory authorities. During *in vivo* pre-clinical studies, QT interval could be assessed directly from recorded electrocardiograms in conscious or in anaesthetised dogs. Nevertheless, the correction factors for heart rate are critical for an accurate and well understood QT interval assessment and the literature suggests that the best formulae to correct QT in dog are those with a minimal correlation with the heart rate. Our laboratory introduced the correction of QT interval as a standard method in pre-clinical dog studies using several different formulae for correction. The electrocardiograms were obtained from 142 (71 males and 71 females) untreated conscious dogs, 24 - 30 weeks old, held in right lateral recumbency. The six main leads (I, II, III, aVF, aVL, aVR) were recorded synchronously and QT intervals were measured from II standard limb leads. We investigated the QT-RR relation in conscious and anaesthetised Beagle dogs by individual analysis of the QT and preceding RR intervals. QTi mean values were 171.8 ms in males and 171.3 ms in females with a range from 140 to 209 ms. The heart rates of both sexes ranged from 73 to 209 beats/min and the RR interval from 287 to 820 ms. QTc were calculated using the Bazett's square root formula, the Fridericia's cubic root formula and the Kovacs' inverted root formula. Results from these studies to address a potential for QT interval prolongation contributed to the design and interpretation of early clinical investigations. The investigation of the potential of pharmaceuticals to prolong the QT interval should be an integral part of pre-clinical safety assessment studies.

1409 A COMPARATIVE HISTOPATHOLOGIC AND METABONOMIC EVALUATION OF VASCULITIS INDUCED BY SK&F 95654, A PHOSPHODIESTERASE III INHIBITOR, IN SPONTANEOUSLY HYPERTENSIVE (SHR) AND NORMOTENSIVE WISTAR-KYOTO (WKY) RATS.

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The present study was initiated to compare the vascular toxicity of SK&F 95654 (SKF) between SHR and WKY. Adult male rats were euthanized 24 h after a single s.c. injection of 100 or 200 mg/kg SKF. SKF caused small vessel vasculitis (with arterial hemorrhage and necrosis) of the mesentery, renal (tubular hyaline droplets, protein casts, glomerular vacuolization), cardiac (necrosis, inflammation, edema), and hepatic (degeneration, inflammation) lesions. Lesions were assessed semiquantitatively microscopically on a scale of 0-5 (cardiac and vascular) or 0-3 (hepatic and renal). Mean scores of vascular lesions were higher in SHR (4.4 and 5.0 given 200 or 100 mg/kg, respectively) than in WKY (0.7 and 0.8 at the same doses). Following dosing with SKF both mast cell prevalence and the percentage of mast cells undergoing degranulation in sections of mesentery increased more significantly in SHR than in WKY. On the other hand, renal lesion scores of 2-3 were seen only in WKY treated with either dose of SKF. Renal pathology correlated with serum elevations in BUN, creatinine, and GGT seen only in WKY. In both strains significant cardiac and hepatic lesions were seen. Cardiac lesion scores of 4-5 were seen in SHR given 200 mg/kg (10/10) or 100 mg/kg (4/6) compared to (2/6) WKY given the high dose of SKF. Hepatic lesion scores of 2-3 were more frequent in WKY, but without changes in serum ALT, AST, or bilirubin. Metabonomic analyses of urine collected by cystocentesis revealed alterations in NMR spectral patterns that segregated by treatment and by the sharply different pathologies produced in the two strains. These results indicate that the differences in SKF-induced toxicities between SHR and WKY are providing mechanistic insight as well as strategies for identifying and evaluating biomarkers associated with drug-induced vasculitis.

1410 POLYCYCLIC AROMATIC HYDROCARBONS WITH BAY-LIKE STRUCTURES INHIBITED GAP JUNCTIONAL INTERCELLULAR COMMUNICATION IN NEONATAL RAT CARDIOMYOCYTES AND CAUSED ASYNCHRONOUS BEATING.

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Cigarette smoke has been implicated in causing or contributing to many human pathologies including cancer and cardiovascular disease. Polycyclic aromatic hydrocarbons (PAHs) are one class of compounds in this complex mixture of combusted

by-products of tobacco that has been extensively studied. Although the most abundant PAHs in cigarette smoke are methylated anthracenes and phenanthrenes, the toxicity of these compounds has not been extensively studied. Recently, several published reports showed that methyl- or chloro-anthracenes, which possess a bay-like structure, induces a release of arachidonic acid, inhibits gap junctional intercellular communication (GJIC), and activates mitogen activated protein kinases in a pleuripotent rat liver epithelial stem cell line. Anthracenes with no bay-like structures were inactive. These biological effects are all molecular events associated with the promotional phases of cancer. In the present study, we demonstrated that anthracenes with bay-like structures inhibited GJIC in primary cultures of cardiac myocytes from neonatal rats, whereas the anthracene lacking a bay-like structure had no affect on GJIC. This inhibition of GJIC directly correlated with the induction of asynchronous beating, illustrating the importance of GJIC in the metabolic coordination of synchronous contraction of cardiac myocytes. These results indicate that a prominent component of cigarette smoke, namely methylantracenes with distinct structural configurations, could be a potential etiological agent contributing to cardiac dysfunction.

1411 VASCULAR SMOOTH MUSCLE CELL PROLIFERATION FOLLOWING CHRONIC, INTERMITTENT EXPOSURE TO 4, 4'-METHYLENEDIANILINE.

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Since 1900, more Americans die annually from cardiovascular diseases (CVD) than from any other disease, and since 1984, more women die from CVD than men. Ironically, few environmental toxicants have been evaluated for their capacities to mediate these diseases. We recently discovered that in female rats weekly, low-dose exposure to 4, 4'-methylenedianiline (DAPM), a compound used in the production of polyurethane, results in vascular medial hyperplasia in both the liver and lung. In addition, treatment of vascular smooth muscle cells (VSMC) in culture with micromolar amounts of DAPM results in VSMC proliferation. Relevant to this newly revealed toxicity, Kautiainen et al. (1998) have suggested that the majority of DAPM metabolite bound to protein in the blood is formed through metabolism by extrahepatic peroxidase enzymes. Peroxidase enzymes metabolize aromatic amines like DAPM to their corresponding imines, which are reactive intermediates capable of binding to DNA and protein. To evaluate whether peroxidase enzymes in VSMC are involved in DAPM-induced VSMC proliferation, we treated VSMC in culture with 0-50 μM DAPM and analyzed the cells for peroxidase activity and the presence of cyclooxygenase-2 (COX-2) protein. Western analysis revealed that VSMC express COX-2, and DAPM treatment increased peroxidase activity 12h after exposure. DAPM or its metabolites thus appear to upregulate the expression of COX-2. Finally, VSMC proliferation was attenuated when cells were simultaneously treated with N-acetylcysteine, a non-protein thiol capable of reacting with electrophilic intermediates, thus competing for their reaction with endogenous molecules and possibly preventing cell damage. The totality of the data presented here suggest that VSMC themselves are capable of metabolizing DAPM to reactive intermediates. (Supported by F32 ES 05892-01.)

1412 TIME COURSE OF PYRIDOSTIGMINE BROMIDE TREATMENT ON BLOOD PRESSURE AND ACETYLCHOLINESTERASE ACTIVITY IN MICE.

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Pyridostigmine bromide (PB) is used primarily for treatment of myasthenia gravis and for prophylactic protection against oranophosphorus nerve poisoning. PB acts by reversible inhibition of acelylcholinesterase (AChE) which is involved in the metabolism of ACh and thus in regulation of neuromuscular and autonomic function. However, there is a little information on the effect of PB on cardiovascular (CV) function. Experiments were performed to determine the time course of the effect of PB on plasma AChE and blood pressure (BP) and heart rate (HR) in conscious mice. The experiment used C57 male mice with chronic carotid arterial catheters for CV measurements and collection of blood samples. PB was administered sc using osmotic minipumps at doses of 1 and 3 mg/kg/day for 7 days. The controls were sham-operated with minipumps filled with saline. BP and HR (sampling rate 85 Hz) were measured for 24 hr on day 0 and days 3 and 7 after minipump insertion. Sequential blood samples (25 ul) for AChE determination were collected on the same days. Mean BP of the control group was 108±5 and 103±4 mm Hg during the dark and light period, respectively. HR was 499± 62 and 478±63 beats/min during the dark and light period, respectively. PB treatment had no effect on BP and HR. Basal AChE activity was 0.44±0.18 umol/min/ml. PB treatment (1 mg/kg/day) had no influence on AChE activity. However, 3 mg/kg/day of PB decreased significantly AChE activity on day 7 by 84±16%. In conclusion, chronic low dose PB exposure in mice decreased AChE activity but had no effect on BP and HR. Supported by DoD contract No.99214005.

1413 TELEMETRY MONITORING OF CARDIOVASCULAR FUNCTION IN CONSCIOUS MALE CYNOMOLGUS MONKEYS AFTER ORAL TERFENADINE OR ASTEMIZOLE.

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A primate telemetry system was evaluated for its utility in detecting QT prolongation, cardiac arrhythmias or other physiological changes after terfenadine or astemizole. Data collection and analysis was done with an Open-A.R.T./Ponemah (Data Sciences International/Gould Instruments) system. A telemetry transmitter designed to monitor blood pressure (BP), core temperature (Temp) and ECG was surgically implanted in each of three male cynomolgus monkeys. The animals were allowed four weeks recovery. Treatments were administered orally (5 mL/kg) at one-week intervals. The dosing sequence was: Control vehicle (0.05% methylcellulose), three doses of terfenadine (30, 100 and 300 mg/kg body weight), control vehicle, three doses of astemizole (10, 30 and 100 mg/kg body weight) and a final control vehicle. Continuous data recording began 2 hours before dosing (0700) and ended 24 hours after dosing (0900 the next day). Animals were recorded on videotape over the same time period (infrared light at night) for subsequent behavior analysis. All parameters were evaluated at half hour intervals before dosing and hourly after dosing. The data files were analyzed for BP, heart rate, QT interval and Temp. QTc (Bazett's) increased after treatment in one of three terfenadine and all three astemizole animals. The QT prolongation was readily detected with the hourly sampling protocol. Five Torsades de pointes (TDP) polymorphic ventricular arrhythmia episodes were detected between 3 and 6 hours after the high astemizole dose in one animal. TDP events were of very short duration (2–4 second) and required continuous monitoring for detection. No other treatment related changes were seen. Heart rate and Temp exhibited consistent diurnal variation during all treatments. In conclusion, the telemetry system and protocol used in this study, successfully detected QT prolongation plus TDP after astemizole and QT prolongation after terfenadine. Continuous recording was needed to insure detection of intermittent polymorphic ventricular arrhythmias.

1414 PREVENTION OF DIABETIC CARDIOMYOPATHY BY METALLOTHIONEIN THROUGH SUPPRESSION OF HYPERGLYCEMIA-INDUCED OXIDATIVE STRESS AND CELL DEATH.

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Diabetic cardiomyopathy is related directly to hyperglycemia; however, proper treatment strategies are unavailable since its cellular and molecular mechanism is unclear. The present study was undertaken to test the hypothesis that hyperglycemia-induced myocardial injury is mediated by oxidative stress and metallothionein (MT), a potent antioxidant, suppress diabetic cardiomyopathy. A cardiac-specific, MT-overexpressing, transgenic mouse model (MT-TG) and non-transgenic controls (WT) were treated with streptozotocin (STZ). About 70 % of the mice treated with STZ-developed diabetes as determined by whole-blood glucose levels. There were no differences in hyperglycemia, body-weight gain, or serum lipid peroxide levels between MT-TG and WT diabetic mice. However, serum creatine phosphokinase activity, a biomarker of myocardial injury, was markedly increased in WT diabetic mice. Correspondingly, myocardial pathological changes as examined by light and electron microscopy and functional alterations as determined by a heart performance analysis were found in WT diabetic mice. Importantly, these diabetic myocardial injuries were all suppressed in MT-TG diabetic mice. In addition, high levels of glucose caused directly lipoprotein and bovine serum albumin (BSA) oxidation, measured by TBARS and Western blotting. High levels of glucose also caused cell death, detected by TUNEL and diapi staining, in cultured cardiac cells. The cell death was associated with accumulation of reactive oxygen species and activation of mitochondrial cytochrome c release-mediated caspase-3 pathway. These results demonstrate that MT prevents diabetic cardiomyopathy, likely through inhibition of cardiac cell death caused by hyperglycemia-derived oxidative stress. (Supported by NIH, AHA and U of L School of Medicine)

1415 DIFFERENTIAL EFFECTS OF LOW AND HIGH CONCENTRATIONS OF QUINIDINE TO INDUCE TORSADE DE POINTES ARRHYTHMIAS: ROLE OF LATE SODIUM AND DELAYED RECTIFIER CURRENTS.

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It is well-known that Torsade de Pointes arrhythmias develop with low, but not high, plasma levels of quinidine. Previous studies have suggested that this may be due to the ability of the drug to amplify transmural dispersion of repolarization (TDR) and thus create a vulnerable window at low concentrations of the drug. Low levels of quinidine were shown to dramatically increase TDR, secondary to a preferential prolongation of the action potential duration (APD) of the mid myocardial (M) cell. Selective block of rapid delayed rectifier current (I_{Kr}) was shown to mediate this action. This study is designed to elucidate the ionic basis for the differential arrhythmogenic effects of low and high concentrations of quinidine. M cells have a smaller slow delayed rectifier current (I_{Ks}) but a larger late sodium current (I_{Na}) compared to epicardial or endocardial cells. We hypothesized that inhibition of I_{Ks} and late I_{Na} underlies the uniform prolongation of APD and the reduction in TDR caused by higher concentrations of quinidine. I_{Ks} was measured using whole cell patch clamp techniques and defined as the chromanol 293B-sensitive tail current elicited by repolarization to 0 mV after a 3 sec step to 40 mV. 5 *micro*|M quinidine did not affect I_{Ks} (1.57 ± 3.7 % inhibition), but 10-25 *micro*|M significantly inhibited the current (79.4 ± 5.9 % inhibition at 25 *micro*|M). Late I_{Na}, (the sustained TTX-sensitive current at the end of a 300 msec depolarizing pulse to 0 mV from -130 mV) was measured using the perforated patch technique. Quinidine (10 and 25 *micro*|M), significantly inhibited late I_{Na}, while lower concentrations were devoid of any effect. In addition to the selective I_{Kr} block observed with low concentrations of quinidine, high levels inhibit I_{Ks} and late I_{Na}. Simultaneous block of all three currents is likely responsible for the homogeneous prolongation of transmural APD and lower arrhythmogenicity of higher levels of the drug.

1416 DOXORUBICIN CARDIOTOXICITY IN SPONTANEOUSLY HYPERTENSIVE RATS.

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Doxorubicin (DOX) is an anthracycline used in the treatment of cancer. Clinical use of DOX has resulted in well documented dose-limiting cardiotoxicity. Using spontaneously hypertensive rats (SHR), we investigated gene expression changes associated with DOX cardiotoxicity in heart, liver and blood. DOX was administered to rats by intravenous injection for up to 2 weeks at a dosage of 1 mg/kg/day, resulting in a cumulative dosage of 1, 3 to 6 mg/kg. Toxicities were evaluated by clinical observation, clinical pathology, light microscopy, and ultrastructural pathology. Dose dependent increase in cTnT levels indicated DOX-induced cardiomyopathy in all treatment groups. This was later confirmed by small multifocal lesions of necrosis observed in all treated groups. Overall 10 genes were regulated in heart, 20 in liver and over 90 in blood. With the exception of a few genes there were few points of overlap in gene expression between treatment groups within a given tissue or between tissues. 12- lipoxygenase was one of these genes and was increased 17 fold in heart, 9 fold in liver and 3 fold in blood in high dose rats. 12-lipoxygenase has been previously implicated for its function in cardiac repair processes and may serve as a marker for DOX-induced cardiotoxicity. Many other genes of interest were regulated in blood after DOX treatment including catalase, transferrin receptor, BCLx, LECAM-1 and others. Further studies are ongoing investigating whether functional correlates ascribed to these transcriptional events correlate with cTnT levels and histopathological changes observed in DOX treated rats. In addition these ongoing studies would help confirm these gene changes and determine their pharmacological or toxicological relevance.

1417 RECRUITMENT OF NUCLEAR RECEPTOR COREGULATORS BY THE PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR α

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The peroxisome proliferator activated receptor α (PPARα) is a member of the nuclear receptor family and regulates genes that are involved in the metabolism of fatty acids in response to peroxisome proliferaters (PPs). Coregulators are a class of proteins whose main function is to enhance or repress the transactivation of genes that are regulated by receptors such as PPARα. In these studies, we examined the nuclear protein p35srj, the RNA coregulator SRA and the PPAR binding protein (PBP). SRA and p35srj were both able to enhance transcriptional activity of PPARα and can be classified as coactivators of PPARα. PBP is a known coactivator for PPARα, leading to enhanced activation of a PP reporter gene. Activation of PPARα by conjugated linoleic acid (CLA) in the presence of PBP leads to enhanced activation of a PP responsive gene to a greater extent than Wy-14, 643. This suggests greater recruitment of PBP to the transcription complex upon activation with CLA. The possibility that coregulator recruitment to nuclear receptor transcription complexes may be ligand specific is being examined.

1418 SELECTIVE DRINKING FOR ALCOHOL PREFERENCE IS PRESENTED ON ACETALDEHYDE DEHYDROGENASE 2 (*ALDH2*) GENE TARGETING MICE.

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Aldehyde dehydrogenase 2 (ALDH2) plays a major role in the detoxification of aldehyde. A diminished enzyme activity due to mutation of the *ALDH2* gene is associated with high alcohol sensitivity and a low tolerance in humans. The genomic background distinguishing an alcohol preference and avoidance of various inbred mouse strains are not clear. And there is no animal model for human *ALDH2* alleles. We genearated *Aldh2* mutant C57BL/6 mice by the targeting knockout, and Aldh2 activity was null in the mitchondrial fractions of the liver of the *Aldh2* -/- mice (K Kitagawa et al. 2000). The mice showed selective drinking for alcohol as like a human *ALDH2* genotype. The *Aldh2* -/- mice consumed only one-third the volume of ethanol solution compared to oridinal *Aldh2* +/+ mice. No significant difference were observed in the body weight and food intake between the two strains during the experiment. The *Aldh2* +/+ and *Aldh2* -/- C57BL/6 mice own same genetic background except *Aldh2*. Therefore, the present study demonstrates a clear effect of *Aldh2* genotype on alcohol preference.

1419 *IN VIVO* QUANTIFICATION OF SEX-SPECIFIC HEME OXYGENASE-LUCIFERASE (HO-LUC) TRANSGENE EXPRESSION FOLLOWING CHLOROFORM-INDUCED TOXICITY IN MICE.

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Heme oxygenase-1 (HO) is a key enzyme in the conversion of heme to bilirubin and may be involved in some cellular anti-oxidative responses. The HO gene has been previously shown to be up-regulated very quickly after treatment with chemicals or other inducers of oxidative stress. FVB mice transgenic for the luciferase gene under transcriptional control of the HO promoter (HO-luc) have been shown to express the HO-luc transgene similarly to the endogenous HO gene following the administration of HO-inducing compounds. *in vivo* tissue-specific expression of HO-luc can be visualized using a low-light imaging system (IVIS ™) after i.p. injection of luciferin. The HO-luc mouse has previously been shown to express luciferase in response to cadmium chloride, a potent HO inducer. In this study, the sex-specific differences in HO-luc expression following chloroform treatment were explored. Male and female HO-luc mice were treated with 100, 200 and 250 mg/kg chloroform either orally or i.p. Male mice showed renal expression of HO-luc whereas female mice did not. Female mice that had been pretreated with testosterone for 3 days prior to chloroform treatment showed HO-luc expression similar to males. This expression pattern was reversed in the same female mice dosed with chloroform 7 days after testosterone depletion. Pathology confirmed the tissue-specific toxicity of chloroform. These findings suggest an androgen-mediated pathway involved in sex-specific chloroform toxicity.

1420 EXPOSURE TO LEAD INCREASES THE ACTIVITY OF SP1-DRIVEN SV40 PROMOTER IN TRANSFECTED PC12 CELLS IN A CONCENTRATION-DEPENDENT MANNER.

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Transcription factors organize and orchestrate differential gene expression, a phenomenon that allows cells to obtain different recipes from the same inherent genome. Zinc finger proteins (ZFP), a class of transcription factors; require Zn for their structural and functional integrity. Hence, ZFPs are susceptible to coexisting heavy metals such as lead (Pb). Sp1, a member of the ZFP family, has been shown to be a cellular target and mediator for Pb-induced anomalies in the regulation of gene expression. Sp1 is a component of a transcriptional complex that is involved in

the regulation of a variety of genes responsible for differentiation and development. It has been shown that Pb modulates Sp1-DNA binding both *in vivo* and *in vitro*. In order to understand the mechanisms involved in Pb-induced interferences at the transcriptional level, we transfected PC12 cells with the vector pGL3 (Promega), which contains a SV40 promoter recognized by Sp1, and a luciferase reporter gene. Twenty-four hours post-transfection, cells were exposed to four concentrations of Pb acetate (0.01uM to 10uM) and nerve growth factor (NGF 50ng/mL). Luciferase activity was measured at 48 hour, post-exposure. NGF increased the activity of the SV40 promoter consistent with its ability to elevate Sp1 DNA-binding as evidenced by the EMSA studies. Very similar to the growth signal NGF, Pb exposure resulted in a consistent increase in SV40 promoter activity that was observed in a concentration-dependent manner. Pb (0.01uM and 0.1uM) resulted in a modest increase when compared to 1uM and 10uM, which resulted in up to a two-fold increase. To confirm the involvement of Sp1 in the activation of the SV40 promoter, we blocked the Sp1 mRNA by co-transfecting with Sp1 antisense oligonucleotides. The activity of SV40 was reduced following such treatment. These data suggest that both NGF and Pb can modulate the function of Sp1-driven transcriptional constructs and gene expression may be perturbed during development through interference with the transcription factor Sp1.

1421 DIFFERENTIAL EFFECTS OF 17β-ESTRADIOL ON VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR EXPRESSION IN ENDOMETRIAL AND BREAST CANCER CELLS LINES.

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Vascular endothelial growth factor receptor (VEGFRII) plays a key role in VEGF-induced angiogenesis. This study investigated the effects of E2 on transcriptional activation of reporter gene (luciferase) activity in HEC1A cells transfected with the following constructs containing VEGFRII gene promoter inserts: -716 to +268 (pVEGFR-A); -225 to +268 (pVEGFR-B); -95 to +268 (pVEGFR-C). In HEC1A cells cotransfected with pVEGFR-A and 250 ng of human estrogen receptor α (hERα) expression plasmid, E2 decreased luciferase activity by 50%, and similar results were observed after transfection with pVEGFR-B and pVEGFR-C. E2-mediated downregulation was also observed in MCF-7 human breast cancer cells, but not in ZR-75 breast cancer cells where E2 induced luciferase activity. Further deletion of the VEGFRII gene promoter indicates a region from -60 to -37 that is important for E2-mediated downregulation in MCF-7 cells and E2-mediated upregulation in ZR-75 cells. This region contains two Sp1 sites and mutation of either site alone or both sites results in the loss of the E2-mediated responses in both cell types. E2-mediated activation and downregulation of reporter gene activity in ZR-75 and HEC1A/MCF-7 cells, respectively, was reversed after cotreatment with the antiestrogen ICI 182, 780. When HEC1A and MCF-7 cells were cotransfected with pVEGFR-C and HE11, a DNA binding domain (DBD) deletion mutant of ERα, E2-dependent downregulation of this promoter was not observed. This indicated that E2-mediated downregulation of pVEGFR-C in HEC1A and MCF-7 cells is dependent on the ERα DBD. In contrast, HE11 was functional for hormone-mediated induction in ZR-75 cells. Current studies are focused on identifying *cis*-acting promoter elements and *trans*-acting factors required for cell-specific modulation of VEGFRII expression. (Supported by NIH CA76636 and ES09106)

1422 MECHANISM OF HORMONAL REGULATION OF *cad* GENE EXPRESSION IN BREAST CANCER CELLS.

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The multifunctional *cad* gene (carbamoyl phosphate synthase, aspartate transcarbamylase, dihydroorotose) regulates pyrimidine nucleotide biosynthesis and is induced by 17β-estradiol (E2) and insulin-like growth factor 1 in estrogen receptor (ER)-positive MCF-7 and ZR-75 breast cancer cells. E2 induced reporter gene activity in MCF-7 and ZR-75 cells transfected with pCAD1 which contains a -90 to +115 *cad* gene promoter insert. This region of the promoter contains two upstream GC-rich motifs that bind Sp proteins and two downstream E-boxes that are important targets in some cells for binding mitogen-induced c-*myc*. In MCF-7 cells transfected with pCAD1, E2 induced reporter gene activity in cells cotransfected with wild-type ERα, ER-11C (DNA binding domain deletion) and ER-TAF1 (D538N, E542Q and D545N mutations that inactivate activation function 2) but not ERβ or ERα mutants that delete N-terminal or C-terminal activation functions 1 or 2, respectively. Moreover, in MCF-7 cells transfected with pCAD1 and a construct expressing a dominant negative form of Sp1 protein, there was complete inhibition of E2-induced transactivation. Deletion analysis of the *cad* gene promoter showed that only the GC-rich and not the E-boxes were required for E2-responsiveness in MCF-7 cells. In contrast, similar studies in ZR-75 cells demonstrated that both GC-rich and E-box motifs were independently E2-responsive indicating significant cell context-dependent differences in hormonal regulation of *cad* even among ER-positive breast cancer cells. The indirect antiestrogen 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin blocks hormone activation of *cad* in both cell lines and the mechanism of this response is being investigated. (Supported by NIH CA64081, ES04176 and ES09106)

1423 MECHANISM OF ESTROGEN RECEPTOR α/Sp1-MEDIATED ACTIVATION OF ESTROGEN-RESPONSIVE GC-RICH PROMOTERS.

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Estrogen receptor α (ERα)/Sp1 activates GC-rich gene promoters in MCF-7 or MDA-MB-231 breast cancer cells, and this response is dependent on activation function 1 (AF1) of ERα. This study investigates contributions of the DNA binding domain (DBD) and AF2 and also promoter context on ERα/Sp1 action in breast cancer cells. In transient transfection studies, wild-type human (hERα) and mouse ERα activated GC-rich constructs containing three tandem Sp1 binding sites ($pSp1_3$) or estrogen-responsive GC-rich promoter inserts from the adenosine deaminase (pADA) or retinoic acid receptor α1 (pRARα1) genes. The pattern of activation was similar for all three promoters in MCF-7 and MDA-MB-231 cells, and binding of hERα and Sp1 to the endogenous adenosine deaminase and retinoic acid receptor α1 gene promoters was observed in chromatin immunoprecipitation assays. The relative contributions of the DBD and AF2 regions of ERα on ERα/Sp1-mediated transactivation were determined using human and mouse ERα constructs containing critical deletions (zinc fingers 1 or 2) and mutations (AF2:D538A, E542A and D545A). The results indicate that the DBD of hERα was not required for estrogen-dependent activation of hERα but was essential for antiestrogen-responsiveness. Results obtained with AF2 mutants and competition with nuclear receptor box (LXXLL) peptides from Grip1 indicate that AF2 of ERα was not essential for ERα/Sp1 action, and prototypical AF2-interacting coactivators did not enhance the activity. Thus, the mechanism of ERα/Sp1-mediated gene expression is uniquely AF1-dependent and differs significantly from the classical ERα activation of an estrogen-responsive element promoter or ERα/AP1 modulation of AP1-dependent promoters/genes. (Supported by NIH CA76636 and ES09106)

1424 SARIN CAUSES DIFFERENTIAL ALTERATION OF MRNAS CODING FOR GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP), VIMENTIN AND ALPHA TUBULIN IN THE CENTRAL NERVOUS SYSTEM OF RATS.

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We studied the effect of a single (50 μg/kg, im) dose of sarin (0.5XLD 50) on the expression of the cytoskeletal genes, GFAP, vimentin (astroglial markers), and alpha tubulin (mostly neuronal) in the central nervous system (CNS) of male rats which were sacrificed at different time points, i.e., 1, and 2 hrs, as well as, 1, 3 and 7 days post-treatment. Northern data collected indicate differential / spatial / temporal regulation of all three transcript levels. Both GFAP and vimentin were induced at 1 hr in all tissues except for brainstem. Overexpressed transcript levels of GFAP and vimentin remained high in more sensitive regions like brainstem and midbrain, while other regions like cortex, cerebellum and spinal cord showed more downward trend for either or both GFAP and vimentin at 7 days. Observed changes in these transcript levels may indicate injury-response and recovery of astroglia. The immediate induction levels of the alpha tubulin transcripts ranged from small (127% in cortex) to high (151% midbrain) except the brain stem, where there was no induction until day one. The levels, however, showed time-dependent increase and reached peak values (246%) at 7 days in the brainstem. The cerebellum showed a great degree of fluctuations in alpha tubulin transcript levels after immediate induction while the cortex showed more or less the same moderately induced levels for the major part of the time course. Immediate induction and variable persistence of these transcripts in sarin-treated CNS suggest that sarin-induced neurotoxicity is in part mediated by the altered expression of cytoskeletal genes. Furthermore, our data indicate that although sarin induces acute toxicity by disrupting the cholinergic system, the development and persistence of long-term neurological deficits may depend on other pathways in the CNS. Supported in part by US .Army Medical Research and Materiel Command under contract DAMD 17-98-C-8027.

1425 VITAMIN D-INTERACTING PROTEIN 150 (DRIP 150) AS A COACTIVATOR OF ESTROGEN RECEPTOR α (ERα)- AND ERα/Sp1-MEDIATED TRANSACTIVATION IN ZR-75 CELLS.

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Vitamin D interacting protein 150 (DRIP 150) is a member of a family of nuclear proteins that interact with a variety of transcription factors and nuclear receptors including estrogen receptor α (ERα). The effects of DRIP 150 as a coactivator of ERα- and ERα/Sp1-mediated transactivation was investigated in ZR-75 breast cancer cells transfected with constructs containing multiple copies of estrogen response elements (ERE) ($pERE_3$) or GC-rich ($pSp1_3$) motifs. In transient transfection studies, DRIP 150 induced a maximal 10- and 4-fold enhancement of estrogen-dependent activation of $pERE_3$ and $pSp1_3$, respectively. The domains of ERα required for DRIP 150-mediated coactivation of ERα and ERα/Sp1 were investigated by cotransfection of inhibitory polypeptides the competitively interact with activation function (AF1) (pAF1) or AF2 (pNR-box). The results showed that ERα/Sp1-mediated transactivation was primarily AF1-dependent, whereas DRIP 150 coactivation involved AF2 of ERα. The following deletion mutants of DRIP 150 have also been generated and these include: DRIP 150m1 (deletion of aa 1145-1454), DRIP 150m2 (deletion of aa 789-1454), and DRIP 150m3 (deletion of aa 325-1454). The results of coactivation of ERα/Sp1 with wild-type and deletion mutants of DRIP 150 indicate that amino acids 789-1145 are required for activity. Current studies are further defining the minimum sequences within DRIP 150 that are necessary for coactivation of ERα/Sp1- and ER-mediated transactivation. (Supported by NIH CA76636 and ES09106)

1426 PROTEIN INHIBITOR OF STAT-1 (PIAS) AS COACTIVATORS OF ESTROGEN-INDUCED GENE EXPRESSION IN BREAST CANCER CELLS.

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The zinc finger region of the androgen receptor has been used as bait to investigate interacting proteins that may subsequently play a role in modulating gene expression. Research in this laboratory has demonstrated that one of these interacting proteins (SNURF) coactivates estrogen receptor α (ERα)- and ERα/Sp1-mediated transactivation in breast cancer cells transfected with constructs containing estrogen response elements ($pERE_3$) and GC-rich ($pSp1_3$) promoter inserts. Protein inhibitor of activated STAT-1 (PIAS) has also been identified as an androgen receptor interacting protein (ARIP) that binds the DNA binding domain, and we have investigated the activity of PIAS proteins as coactivators of ERα and ERα/Sp1 in breast cancer cells. E2 induces reporter gene activity in ZR-75 cells transfected with $pERE_3$ and in cells cotransfected with 5 - 300 ng rat ARIP3 (homologous to human PIASxα), there was a > 3-fold coactivation using 50 ng ARIP3 expression plasmid, and significant coactivation was observed using up to 300 ng ARIP3. In contrast, ARIP3 coactivated ERα/Sp1-mediated transactivation only 1.7-fold in ZR-75 cells over a smaller range of expression plasmid concentration (5 - 50 ng). In parallel studies, the coactivator activities of human PIAS1 and PIAS3 were also investigated in ZR-75 cells transfected with $pSp1_3$. The results showed that PIAS1 was inactive as a coactivator, whereas PIAS3 caused a 2.4-fold enhancement of ERα/Sp1-mediated activation of $pSp1_3$. Current studies are focused on the mechanisms of PIAS proteins as coactivators of ERα and ERα/Sp1 and domain-specific physical and functional interactions of ERα and Sp1 with PIAS proteins. (Supported by NIH CA76636 and ES09106)

1427 TYROSINE AND SERINE-THREONINE KINASE INHIBITORS BLOCK SODIUM ARSENITE INDUCED DISRUPTION OF THE ACTIN CYTOSKELETON AND FOCAL ADHESIONS.

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Arsenic is a human carcinogen, but the diverse acute physiological effects caused by arsenic are likely due to the activation of various signaling pathways rather than genotoxicity. We used immuno-fluorescence microscopy to study the effects of 0.25-25 micromolar sodium arsenite on the actin cytoskeleton, focal adhesions, and mitochondrial localization in mouse fibroblasts. At 0.25 micromolar, short-term effects of sodium arsenite were minimal, but at higher concentrations we noted loss of F-actin and vinculin and a concomitant change in cell morphology, from flat and well-spread to rounded and loosely attached. At 25 micromolar, sodium arsenite caused a severe loss of F-actin and vinculin and most cells detached from the substrate. Signaling by tyrosine and serine-threonine phosphorylation play important roles in regulation of the actin cytoskeleton and focal adhesion formation. Co-treatment with the tyrosine kinase inhibitor genistein or the serine-threonine kinase inhibitor staurosporine completely blocked these toxic effects of sodium arsenite. Studies currently underway will identify the specific members of these kinase families that appear to mediate the toxic effects of sodium arsenite.

1428 COCAINE INDUCES A DOSE DEPENDENT ALTERATION IN THE EXPRESSION IMMEDIATE EARLY GENES *C-FOS* AND SP-1 AND IN NUCLEAR FACTOR NF-Kß IN PC12 CELLS.

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Cocaine is a widely used drug of abuse and psychostimulant which acts on the central nervous system by blocking the dopamine re-uptake sites. PC12 cells, a rat pheochromocytoma clonal line, which in the presence of nerve growth factor (NGF), multiply and differentiate into competent neurons that can synthesize, store and secrete the neurotransmitter dopamine (DA). In the present study, we evaluated the effect of increasing doses of cocaine on the expression of immediate early genes (IEGs), *c-fos* and SP-1 and a closely related nuclear factor, NF-Kß 24 h after the exposure to cocaine (50, 100, 250, 500, 1000, 2500 µM) in NGF-differentiated PC12 cells. Cocaine (50-500 µM) resulted in significant induction of the expression of *c-fos*, SP-1 and NF-Kß. However, higher concentrations of cocaine (1000 & 2500 µM) resulted in the down-regulation of these expressions after 24 h. We also evaluated the effect of increasing doses of cocaine on dopamine. A significant dose-dependent decrease in the concentration of dopamine was observed 24 h after the exposure of PC12 cells to cocaine. Therefore in the present study, we reported that cocaine has both upstream and downstream regulatory actions on some IEGs and nuclear factors which is independent of its action on the dopaminergic system. Further studies are underway to explore more mechanistic details.

1429 INDUCTION OF *p53* GENE EXPRESSION IN MCF-7 BREAST CANCER CELLS BY 17β-ESTRADIOL IS MEDIATED BY CALMODULIN KINASE IV-DEPENDENT ACTIVATION OF AN NF-κB/CTF-1 COMPLEX.

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Initial studies in this laboratory showed that 17β-estradiol (E2) induced tumor suppressor gene *p53* mRNA and protein levels in MCF-7 human breast cancer cells. Analysis of the *p53* gene promoter identified a minimal E2-responsive region (-106 to -30) that binds multiple transcription factors, but only motifs that bind CTF-1 and NF-κB are required for hormone-induced activation of p53-10 (contains a -106 to -30 *p53* gene promoter insert). The p65 subunit of NF-κB was identified in both nuclear and cytosolic fractions of untreated MCF-7 cells, and induction of *p53* by E2 was inhibited by the IκB super repressor and precursor-derived inhibitor containing the C-terminal region of p105, the precursor of p50. Although nuclear p65 is required for hormonal activation of *p53*, formation of nuclear NF-κB complex was essentially E2-independent. We therefore investigated direct activation of *p65* in MCF-7 cells in transient transfection studies using p53-10 and a chimeric protein containing the C-terminal 30 amino acids of *p65* (pM-p65c2) fused to the yeast GAL4 protein DNA binding domain. Results of studies using selective kinase inhibitors showed that only KN-93, an inhibitor of calmodulin kinase IV (CaMKIV), blocked hormone activation of p53-10 or pM-p65c2, and inhibition was also observed with ethylene glycol tetraacetate-AM, a cell permeable chelator of divalent calcium ions. In addition, constitutively active CaMKIV also activated pM-p65c2 in MCF-7 cells. Our results demonstrate that *p53* is upregulated by E2 in MCF-7 cells through direct kinase-dependent activation of *p65* and cooperative p65-CTF-1 interactions. (Supported by NIH ES09255 and ES09106)

1430 ANALYSIS OF ESTROGEN RECEPTOR α (ERα) AND Sp1 PROTEIN INTERACTIONS WITH PROMOTERS OF ESTROGEN RESPONSIVE GENES IN BREAST CANCER CELLS BY CHROMATIN IMMUNOPRECIPITATION.

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Studies in this laboratory have identified GC-rich elements in the promoters of the cathepsin D (CD), retinoic acid receptor α1 (RARα1), and adenosine deaminase (ADA) genes that bind estrogen receptor α (ERα)/Sp1 *in vitro* and are required for

estrogen (E2)-induced transactivation. Interactions of ERα, Sp1 and other nuclear transcription factors with specific E2-responsive regions of the CD, RARα1 and ADA gene promoters were investigated by the chromatin immunoprecipitation (ChIP) assay. Briefly, MCF-7 human breast cancer cells were treated with 10 nM E2 for 30, 60, 90 or 120 min; formaldehyde was then added to crosslink proteins and DNA, isolated nuclei were obtained and sonicated for 45-60 sec to obtain chromatin with appropriate fragment length (500-1000 bp). ERα and Sp1 antibodies were used to immunoprecipitate chromatin associated with these proteins and, after removal of the crosslinks and extensive purification, DNA from immunoprecipitated fractions was analyzed by PCR with primers to detect E2-responsive regions of the CD (-246/+17), RARα1 (-187/-10) and ADA (-148/+51) gene promoters. PCR analysis demonstrated that both ERα and Sp1 proteins occupied the CD gene promoter for at least two hours after treatment with E2 and, in contrast to previous reports, cyclic occupation of the promoter by these proteins was not detected. Using a similar approach, we also observed occupation of the ADA and RARα1 gene promoters for up to 120 min by both Sp1 and ERα proteins; however, there was some variability in ERα-promoter interactions that was dependent on the antibody used in the immunoprecipitation step. Current studies are refining this technique, extending the time-course studies, and determining interactions of other coactivator proteins with these gene promoters. (Supported by NIH CA76636 and ES09106).

1431 COOPERATIVE COACTIVATION OF ESTROGEN RECEPTOR α IN ZR-75 HUMAN BREAST CANCER CELLS BY SNURF AND TATA-BINDING PROTEIN.

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SNURF is a small RING finger protein that binds the zinc finger region (ZFR) of steroid hormone receptors and enhances Sp1- and androgen receptor-mediated transcription in COS and CV-1 cells. In this study, we show that SNURF coactivates both wild-type estrogen receptor α (ERα) (4-fold) and HE19 [ERα deletion of activation function 1 (AF1)] (10-fold)-mediated activation of an estrogen responsive element (ERE) promoter in ZR-75 cells. In mammalian two-hybrid assays conducted in ZR-75 cells, SNURF interactions with ER were estrogen (E2)-dependent and not observed with the antiestrogen ICI 182, 780. ERα interacted with multiple regions of SNURF; SNURF interactions with ERα were dependent on the helix 12 of AF2, since D538N, E542Q and D545N mutations in the region abrogated both SNURF-ERα binding and coactivation. Moreover, peptide fusion proteins that inhibit interactions between helix 12 of ERα with LXXLL box-containing proteins also blocked ERα coactivation by SNURF, suggesting that confactors that interact with helix 12 are required for coactivation by SNURF. However, cotransfection of SNURF with prototypical steroid receptor coactivators 1, 2 and 3 that contain LXXLL box motifs did not enhance E2-responsiveness, whereas TATA binding protein (TBP) cooperatively coactivated ERα-mediated transactivation. The results are consistent with a unique model for cooperative coactivation of ERα that requires ligand binding, repositioning of helix 12, recruitment of TBP, and interaction with SNURF. (Supported by NIH CA76636 and ES01906)

1432 *IN VIVO* ANALYSIS OF ESTROGEN-INDUCED CYCLIN D1 EXPRESSION UTILIZING *IN SITU* HYBRIDIZATION AND CHROMATIN IMMUNOPRECIPITATION (ChIP) ASSAYS IN A MURINE MODEL.

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Cyclin D1 (CD1) is an important protein in the normal development of the reproductive tract and mammary glands and in the development of mammary carcinogenesis. 17β-Estradiol (E2) induces CD1 expression in estrogen receptor (ER)-positive human breast cancer cells; the promoter elements involved are cell context-dependent. For example, a GC-rich Sp1-binding site, activated by ERα/Sp1 complexes, is functional in ZR-75 but not in MCF-7 cells. Utilizing the mouse uterus model, we compared a time course for CD1 mRNA expression in the uterus of wild-type and estrogen receptor α knockout (ERKO) mice treated with E2. In wild-type mice, CD1 mRNA levels were induced by E2 in the luminal epithelium of E2-treated mice after 6 hr; however, in ERKO mice, the staining of CD1 mRNA was comparable in the E2-treated and control mice at 6 hr. Thus, ERα is necessary for the induction of CD1 by E2 in the mouse uterus. Furthermore, to confirm the interaction of ERα with both the murine and human CD1 gene promoters *in vivo*, we adapted the ChIP assay for analysis of freshly collected murine tissue and xenografts of human breast cancer cells in mice. Cells were isolated, lysed by hypotonic bursting, and isolated nuclei were obtained and sonicated to obtain chromatin with appropriate fragment length (500-1000 bp). ERα and Sp1 antibodies were used to immunoprecipitate chromatin associated with these proteins, and the chromatin was analyzed by PCR with primers to detect E2-responsive regions of the human CD1 (-204/-103) and the murine CD1 (-156/+7) gene promoters. Using this technique, we have shown that Sp1 is bound to the CD1 promoter in mouse uterine cells and ERα is bound to the CD1 promoter in brain and liver cells. The ChIP assay will be utilized along with *in situ* hybridization analysis of other E2-responsive genes in the mouse to determine the presence of ERα, Sp1, and other transcription factors on their respective promoters. (Supported by NIH CA70636 and ES09106)

1433 VITAMIN D INTERACTING PROTEIN 205 (DRIP 205) AS A COACTIVATOR OF ESTROGEN RECEPTOR α/Sp1-MEDIATED TRANSACTIVATION.

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Several independent studies have identified vitamin D interacting protein 205 (DRIP 205) as one of a family of mediator complex proteins that associate with transcription factors and hormone receptors. DRIP 205 coactivates 17β-estradiol (E2)-induced gene expression in ZR-75 and other breast cancer cell lines transfected with wild-type estrogen receptor α (ERα) and constructs containing estrogen response elements ($pERE_3$) or GC-rich motifs ($pSp1_3$) that bind ERα/Sp1. Ten nM E2 alone induced reporter gene activity in ZR-75 cells transfected with $pSp1_3$ and wild-type ERα (HE11) (a DNA binding domain deletion mutant) or hER-TAF1 [point mutations inactivating activation function 2 (AF2)]. Cotransfection with DRIP 205 coactivated wild-type/mutant ERα 3.8-, 2.7- and 1.7-fold, respectively. DRIP 205 contains 1566 amino acids including two nuclear receptor (NR)-boxes containing LXXLL motifs (within the aa 528 - 714 sequence) that interact with helix 12 in the AF2 domain of ERα. Plasmids expressing aa 1 - 714 (DRIP 205m1), aa 516 - 1566 (DRIP 205m2), and aa 528-714 (DRIP 205m3) were prepared by PCR. The coactivator activity of mutant DRIP 205 expression plasmids was investigated in ZR-75 cells transfected with $pSp1_3$ and ERα. Both N-terminal (DRIP 205m2) and C-terminal (DRIP 205m1) deletion mutants of DRIP 205 coactivated ERα/Sp1-mediated transactivation 3.6- and 2.0-fold, respectively, whereas DRIP 205m3 was inactive. Current studies are focused on delineating specific domains of DRIP 205 required for coactivation of ERα/Sp1. (Supported by NIH CA76636 and ES09106)

1434 THE UTILITY OF LARGE-SCALE INSERTIONAL MUTAGENESIS (GENE TRAP) SCREENS TO IDENTIFY AND DISRUPT NOVEL GENES INDUCED BY TCDD IN MOUSE EMBRYONIC STEM(ES)CELLS.

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Polychlorinated dibenzo–*p*–dioxins (PCDDs) are industrial compounds or by–products which have been identified in extracts from diverse environmental matrices including fish, wildlife and humans. PCDDs are complex mixtures of congeners with a limited number being ubiquitous in environmental samples. Recently, PCDDs have been the focus of extensive toxicological studies and serious public debate. As a result of this, 2, 3, 7, 8–tetrachlorodibenzo–*p*–dioxin (TCDD) has been identified as the most toxic congener in the PCDD family and has been shown to elicit numerous adverse responses in animals including neoplasia, immunosuppression, hepatotoxicity, neurotoxicity, reproductive toxicity, and teratogenesis. While TCDDs physiological effects are well known in animals, the overall risk that TCDD poses to humans is unclear. Moreover, outside of the "classical dioxin-inducible gene battery" little is known about the complex molecular actions of TCDD, which are likely to be associated with many observed physiological effects. To address this issue, we have developed an experimental strategy that simultaneously identifies TCDD-inducible genes, mutates them, tags them with a reporter molecule, and enables the production of knockout animals carrying the induced mutations. This process has been termed "gene-trapping." The power of this technology is the ability to discover novel genes responsive to a particular chemical, rather than testing the responsiveness of a known gene. Within the scope of this research, we have conducted the molecular and functional characterization of the role of these genes in cells and animals. To date, 26 TCDD-sensitive genes have been identified out of approximately 2000 mutated genes; 12 of those genes have been sequenced. The generation of a knockout mouse for one identified gene is currently in progress.

1435 TRAPPING GENES ASSOCIATED WITH TOBACCO USE.

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Data from epidemiological studies have strongly linked the development of squamous cell carcinomas of the upper aerodigestive tract to consumption of tobacco, particularly when combined with alcohol. The exact mechanisms leading from to-

bacco use to disease are not well understood, but for tumorigenesis those mechanisms are surely genetic. Understanding how carcinogens induce tumor formation ultimately relies on identifying and characterizing the genes that are targeted for mutation and/or dysregulation by those carcinogens. To this end, many mutations have been found in cancer cells from tobacco users, but not all mutations have been associated with specific genes. Thus, the goal of this project is to identify, mutate and characterize those genes. This goal is part of the long-term objective of understanding the genetic mechanisms underlying the development of cancer. To address this goal we have deployed a gene-trapping strategy to identify tobacco-sensitive genes. Briefly, mouse embryonic stem (ES) cells are transfected with a promoterless reporter gene construct. Successful transfection of an ES cell will knockout an endogenous gene, but the reporter gene will be active and under the control of the endogenous promoter. Large numbers of unique gene-trapped ES cell clones are then screened in the presence or absence of cigarette smoke condensate (CSC), and altered reporter gene expression is identified and recorded. The reporter gene itself is then used as the starting point for sequencing of the endogenous genes. Sequences are then compared to those in the GenBank database to identify novel genes as well as previously known genes that were unknown to be altered/dysregulated by tobacco. In addition, mutant ES cells created as described above can be used to generate knockout mice. Those mice, in turn, will then be studied to understand the role of each gene relative to the development of cancer and disease.

1436 PREGNANE X RECEPTOR MEDIATES THE INDUCTION OF RAT ORGANIC ANION TRANSPORTING POLYPEPTIDE 2 BY PREGNENOLONE-16 ALPHA-CARBONITRILE.

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The rat organic anion transporting polypeptide 2 (oatp2; Slc21a5) is a hepatic sinusoidal transporter that mediates the uptake of a variety of structurally diverse compounds, and has a very high affinity for cardiac glycosides. Treatment of rats with pregnenolone-16 alpha-carbonitrile (PCN), a potent ligand for the rodent pregnane X receptor (PXR), significantly enhances the transcription of the oatp2 gene leading to subsequent increases in hepatic oatp2 mRNA and protein levels. In an effort to understand the molecular mechanism of oatp2 induction by PCN treatment, approximately 8700 bp of the 5' flanking region of the rat oatp2 gene were linked to the luciferase reporter gene and used in transient transfection assays in H4IIE cells. PCN treatment induced the expression of the reporter gene in a dose-dependent manner. A total of four potential PXR response elements (PXRREs) were identified in the 5' flanking region of the rat oatp2 gene. One element (DR3-1) is located approximately -5000 bp with three more (DR3-2, DR3-3, DR3-4) clustered at about -8000 bp upstream of the predicted transcription start site. Results gathered from Electrophoretic Mobility Shift Assays showed that the PXR-RXRalpha heterodimer directly binds to the DR3-2 with the highest affinity, followed by to the DR3-4 and DR3-1, but only to the DR3-3 weakly or not at all. Furthermore, a series of partial deletions of the 5' flanking region of the rat oatp2 gene illustrated that both the proximal and distal cluster of PXRREs are required for maximal induction of rat oatp2 by PCN. In conclusion, these data elucidate the molecular mechanism by which PCN treatment induces rat oatp2 gene expression. In addition, this study identifies rat oatp2 as a direct PXR-targeted gene and further supports the hypothesis that activation of PXR affects a network of genes that is involved in either metabolism or transport of drugs, steroids, and bile acids. (Supported by NIH grant ES-09649 and ES-07079)

1437 ENDOCRINE REGULATION OF RAT ORGANIC ANION TRANSPORTER 2 (OAT2) mRNA EXPRESSION IN KIDNEY.

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Initially, rat Oat2 was described as a liver-specific transporter. However, recent data indicate gender divergent expression of Oat2 in which male levels are highest in liver while female levels are highest in kidney. Furthermore, in kidney there was a female-predominant pattern of Oat2 evident after the onset of sexual maturity. Therefore, the goal of this study was to investigate endocrine involvement in the elevation of Oat2 mRNA levels in female as compared to male kidney. Sex steroid influence was determined by comparing Oat2 mRNA levels in kidneys of castrated and ovariectomized rats to those of intact control rats. Additionally, endocrine effects were studied in hypophysectomized (HX) rats randomly placed in one of the following four treatment groups: 1) testosterone (daily); 2) estrogen (daily); 3) male-pattern growth hormone (two injections per day to simulate pulsatile secretion); or 4) female-pattern growth hormone (continuous infusion *via* mini-osmotic pumps). Hormones were administered subcutaneously for 5 days. Renal Oat2 mRNA levels were measured by branched DNA signal amplification. Levels of Oat2 mRNA were 10-fold higher in control female kidneys as compared to control male kidneys. Interestingly, gonadectomy did not affect male or female Oat2 mRNA levels, but HX did decrease renal Oat2 levels in females by 90%, resulting in Oat2 mRNA levels in kidney of HX females equivalent to those in kidneys of control male rats. Neither testosterone nor estrogen administration altered Oat2 mRNA levels in kidneys of HX rats. Likewise, male-pattern growth hormone replacement did not increase Oat2 mRNA levels in kidneys of HX males or HX females. However, continuous infusion of rat growth hormone, which mimics female-pattern secretion, did increase renal Oat2 mRNA levels in both HX male and HX female rats. Therefore, these data demonstrate that the gender difference in Oat2 mRNA levels in kidney is not the result of sex steroid influences, but rather a function of female-pattern growth hormone secretion. (Supported by NIH grants ES-09716 and ES-07079)

1438 TISSUE EXPRESSION OF THE RAT UDP-GLUCURONOSYLTRANSFERASE SUPERFAMILY.

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UDP-Glucuronosyltransferases (UGTs) are a superfamily of phase II drug metabolizing enzymes that catalyze the conjugation of glucuronic acid to a broad spectrum of endobiotic and xenobiotic substrates. The rat UGTs have been divided into two gene families, UGT1 and UGT2, which include at least 14 enzymes. The complete tissue expression of rat UGTs has not been thoroughly examined, but could provide further indication of individual UGT functions. The aim of this study was to quantitatively determine the mRNA levels of rat UGT1 and UGT2 families in various tissues using the branched DNA signal amplification method. UGT1A1 was present at high levels in all tissues examined except lung. UGT1A2 was detected primarily in small intestine. UGT1A5 was detected primarily in liver at low levels. UGT1A6 was present predominantly in kidney and gastrointestinal (GI) tract, whereas UGT1A7 was detected in the GI tract and at low levels in lung. UGT2A1 was detected at high levels only in nasal epithelium. UGT2B1 was detected only in liver. UGT2B3 and 2B6 were detected mainly in liver, whereas 2B8 was detected in stomach, duodenum, and kidney at low levels. UGT2B12 was primarily localized in liver and kidney, with lower levels in duodenum. Finally, UGT1A3, 1A8, and 2B2 were difficult to detect. In summary, the UGTs present in liver include UGT1A1, which was present at high levels, and 2B1, 2B3, 2B6, 2B12, 1A5, and 1A6, which were present at low levels. In both small and large intestine UGT1A1, 1A6, and 1A7 were present at high levels, but 1A2, 2B8, and 2B12 were present in small intestine at low levels. UGT1A1 and 1A6 were present at high levels in kidney, in which lower levels of 2B12 and 2B8 were detected. UGT1A1, 1A6, 1A7, and 2B12 were present at low levels in lung. Only UGT1A1 was present at high levels in brain, with 1A6, 2B3, and 2B12 at low levels. The tissue specific expression of UGTs may be responsible for the tissue specific detoxification or activation of UGT substrates and thus suggests a possible role in target organ toxicity. (Supported by NIH grants ES-09716 and ES-07079)

1439 LIPOPOLYSACCHARIDE-MEDIATED REGULATION OF HEPATIC TRANSPORTER mRNA LEVELS IN RATS.

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The major function of hepatic transporters is to move a wide range of organic substances across the sinusoidal and canalicular membranes. During extrahepatic cholestasis, some hepatic transporters that are involved in the normal vectoral flow of organic substances from blood to bile, such as Ntcp and Mrp2, are known to be dramatically down-regulated, while other transporters such as Mrp3 are up-regulated. Unlike models of extrahepatic cholestasis where alterations in transporter expression are in response to the accumulation of bile constituents in the hepatocyte, lipopolysaccharide (LPS) may directly alter transporter expression leading to cholestasis characterized by impaired bile flow. The aim of this study was to quantitatively determine the effect of LPS on the mRNA levels of hepatic transporters in Sprague-Dawley rats and whether dexamethasone pretreatment could modulate the LPS-mediated changes in hepatic transporter mRNA levels. Sixteen h after LPS (4 mg/kg) administration, the branched DNA signal amplification assay revealed that hepatic mRNA levels of Mrp2, Mrp6, Mdr1a, Oatp1, Oatp2, Lst, Ntcp, Bsep, Oct1, and Oat3 were dramatically decreased, but Mrp5, Mdr2, and Oat2 levels remained unchanged. In contrast, LPS administration increased mRNA levels of Mrp1, Mrp3, and Mdr1b. A 1 h pretreatment with dexamethasone (10 mg/kg) significantly diminished the reduction of Mdr1a, Oatp1, Oatp2, Oct1, and Ntcp mRNA following LPS administration, but had little or no effect on the decrease of Mrp2, Mrp6, Lst, Bsep, and Oat3 levels. Dexamethasone also blocked the LPS-mediated increase in Mrp1, Mrp3, and Mdr1b mRNA. The dexamethasone abatement of both LPS-mediated suppression and induction of transporters indicates

that these responses may be mediated through similar pathways. Moreover, the prevention of LPS-mediated effects by dexamethasone pretreatment on some, but not all transporters underscores the complex nature of hepatic transporter regulation. (Supported by NIH grants ES-09716, ES-09649, and ES-07079)

1440 DOWN-REGULATION OF LIVER-SPECIFIC TRANSPORTER (Lst OR Oatp4) BY LIPOPOLYSACCHARIDE THROUGH TOLL-LIKE RECEPTOR 4 (TLR4).

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Lipopolysaccharide (LPS) is a component in the outer membrane of Gram-negative bacteria. LPS stimulates the immune system and causes a dramatic systemic reaction known as sepsis, which can cause cholestasis in humans and rodents and is associated with the down-regulation of the liver-specific transporter (Lst or Oatp4) and other transporters in rat liver. Biological action of LPS is thought to be mediated by toll-like receptor 4 (TLR4). The purpose of this study was to determine (1) whether LPS treatment decreases the levels of Lst mRNA in mice and (2) whether TLR4 plays a role in the LPS-mediated decrease of Lst expression. To address this hypothesis, C3H/HeJ mice were used because they are LPS-hyporesponsive due to a missense mutation (P712H) in TLR4. LPS-responsive C3H/OuJ mice were used as controls. Both C3H/HeJ and C3H/OuJ mice (25-30 g) were injected with a single, nonlethal dose of 5 mg/kg LPS ip in saline. Livers were excised at various time points: 0, 1.5, 3, 6, 12, 16, 24, and 48 h after treatment, and the levels of Lst mRNA were analyzed by the branched DNA signal amplification assay. In control C3H/OuJ mice, LPS treatment decreased Lst mRNA levels rapidly in a time-dependent fashion, reaching the minimal level (about 80% decrease) at 12 h following treatment, and returning to normal levels over the following 36 h. In the TLR4 mutant mice (C3H/HeJ), the decrease in Lst mRNA levels was much less pronounced (about 40% decrease) than that observed in the controls (C3H/OuJ). A complete ablation of the response to LPS was not expected in the TLR4 mutant mice (C3H/HeJ), because these mice are hyporesponsive but not unresponsive to LPS. These data support the hypothesis that the LPS-mediated decrease in the expression of Lst is through TLR4. (Supported by NIH grant ES-09649)

1441 TISSUE DISTRIBUTION AND REGULATION OF THE MULTI-DRUG RESISTANCE PROTEINS 5 AND 6.

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Multidrug Resistance Proteins (Mrps) are a class of ATP-dependent transporters distinguished by their ability to confer resistance to anti-cancer drugs. Two of the newest family members, Mrp5 and Mrp6, were only recently cloned and have not been well characterized. The purpose of this study was to determine the tissue distribution of Mrp5 and 6, and to ascertain whether hepatic mRNA levels of the transporters were altered by cholestasis or administration of microsomal enzyme inducers. Mrp5 and 6 mRNA levels were quantified using the branched DNA signal amplification method. Transporter mRNA levels were examined in Sprague-Dawley rats for both Mrp5 and Mrp6 in various tissues. Additionally, microsomal enzyme inducers representing six different transcriptional activation mechanisms were utilized to determine effects on Mrp5 and 6 mRNA levels. In a subsequent study, bile-duct ligation was used to model cholestasis, and the transcriptional regulation of Mrp5 and 6 was subsequently examined. Mrp5 mRNA levels were detected predominantly in the adrenal gland, followed by cerebral cortex, cerebellum, and stomach. Expression of Mrp5 in the adrenal gland was substantially higher than that present in other tissues. In contrast, Mrp6 tissue expression was more ubiquitous, but expression was highest in intestinal tissue, liver, and kidney. Mrp5 mRNA expression was minimal in the liver, and neither treatment with microsomal enzyme inducers nor bile duct ligation altered mRNA levels. Mrp6, while present in liver, also showed little alteration by these treatment conditions. In conclusion, Mrp5 is most highly expressed in the adrenal gland, while Mrp6 is mainly expressed in excretory organs (liver, intestine, and kidney), suggesting markedly different functions. Additionally, the hepatic mRNA levels of Mrp5 or Mrp6 are not altered in response to challenges such as bile duct ligation or microsomal enzyme induction. (Supported by NIH grants ES-09716 and ES-07079)

1442 SIGNAL TRANSDUCTION PATTERNS IN VARIOUS MOUSE IMMUNE SURVEILLANCE CELLS AFTER XENOBIOTIC STIMULATION.

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With the exception of stem cells, as a general rule cells are specialized to perform a primary function or limited set of functions unique to its type or subset. Immune cells, as a subset, have functions that are distinguished by the role that particular cell plays in the inflammatory process. A determining factor of this role is based, in part, on the mediators (or endpoints) released from a cell, which regulate the inflammatory response. The activation and release of these endpoints are controlled by complex intracellular signaling mechanisms. A single signaling molecule, such as LPS or PMA, can activate a variety of endpoints (genetic changes occurring at the termination of a signal transduction cascade) in different subsets of immune cells. Multiple signal transduction pathways can be activated to create unique patterns of activation (signature response), which may or may not to be toxicant and cell specific. In order to determine the specificity of this response, alveolar macrophages, spleen dendritic cells, and PMN's (various surveillance cells important in the inflammatory process) were isolated from mice and stimulated with either LPS or PMA. Genomic array analysis of cells stimulated with either LPS or PMA revealed a unique expression profile for signal transduction pathways for each toxicant that was common to all cell types, though endpoints varied considerably among the cell types. These data suggest that intracellular "signature responses" can occur which are unique to the inducing toxicant or class of toxicants, but common to all cells or subset of cells. These "signature responses" can be utilized to develop a mechanistic understanding of toxicity and exploited to develop rapid, cost effective *in vitro* assays. Understanding mechanisms of intracellular signal transduction can lead to improved *in vitro* screening assays which ultimately reduce animal usage and provide less expensive assays for screening potential toxicants.

1443 ALTERNATIVE PROCESSING OF THE HUMAN *FMO6* TRANSCRIPT.

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The flavin-containing monooxygenases (FMO) are important for the metabolic disposition of a wide variety of environmental toxicants and therapeutics. There is evidence for five distinct mammalian flavin-containing monooxygenases (FMO1-5) each exhibiting a highly regulated tissue-, developmental, and species-specific expression pattern. A sixth *FMO* gene sequence is found within the *FMO* cluster on the long arm of human chromosome 1. Although this gene exhibits the expected 48-55% sequence identity with FMO1, FMO2, FMO4 and FMO5, it is unusual in that it shares 70% sequence identity with FMO3. Sequence analysis revealed the presence of the conserved NADPH and FAD binding elements typical of this gene family and predicted normal splicing to yield a 1, 649 base plus transcript encoding a 539 amino acid protein. However there has been no evidence of FMO6 expression or demonstration of specific enzyme activity. In this study, RNA was isolated from human liver and the FM06 cDNA was amplified by nested RT-PCR. Products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. Individual amplification products were purified and sequenced using the PCR primers. The expected cDNA amplification products were not observed in any of the samples. Rather, truncated versions were seen in the range of 1, 100 to 1, 400 bases. Examination of the DNA sequence revealed splice variants which either eliminated or shortened some of the expected exons. Comparison to *FMO3* revealed sequence changes at each of the alternative splice sites conforming to consensus splice donor or splice acceptor sites. Open reading frame analysis of these variant transcripts indicated a loss of competency to encode full length functional enzyme and suggests that FMO6 activity is not present in hepatic tissue and that this gene represents a pseudogene. Further studies will examine *FMO6* splicing scenarios in other tissues. (Supported in part by PHS Grant No. CA53106).

1444 p, p'-DICHLORODIPHENYL DICHLOROETHYLENE (p, p'-DDE) INHIBITS THE ANDROGEN-INDUCED ANTIPROLIFERATIVE RESPONSE IN CAMA-1 CELLS.

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We recently reported that p, p'-DDE, the main metabolite of p, p'-DDT and a potent anti-androgen, was associated with tumor aggressiveness in women diagnosed with breast cancer (Cancer Epidemiol. Biomarkers Prev. 2000; **9**: 161-6). Since androgens are known to inhibit the growth of breast cancer cells, we tested the hypothesis that p, p'-DDE might block this antiproliferative response in the estrogen-responsive CAMA-1 human breast cancer cell line that expresses the androgen receptor. CAMA-1 cell proliferation was determined by seeding 2 x 10^4 cells into 24 well plates and exposing the cells to various concentrations of 17-bestradiol (E2), dihydrotestosterone (DHT) or p, p'-DDE for 12 days. Cells were then fixed with methanol and the effect on cell growth was determined by measuring DNA in the cells by fluorometry, following the addition of DABA. The maximum proliferative response was observed with 0.1 nM E2. DHT added alone to the cell medium did not increase cell proliferation, but 0.1 and 1 nM DHT reduced the proliferative response induced by 0.1 nM E2 by 30% and 50%, respectively. p, p'-DDE had no effect by itself on CAMA-1 cell growth. However, in combination with 0.1 nM E2 and 0.1 nM DHT, increasing concentrations of p, p'-DDE in the incubation

medium (0.1–1 μM) partially restored the proliferative response induced by E2. These results add biological plausibility to our previous epidemiological observations linking p, p'-DDE and breast cancer aggressiveness.

1445 MECHANISM OF P53 ELEVATION BY BUTADIENE DIEPOXIDE IN HUMAN FIBROBLASTS.

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The environmental chemical butadiene is prevalent in petrochemical industrial areas, and is a known mutagen and suspected human carcinogen. The molecular mechanisms of butadiene toxicity, however, are not yet well understood, though the cellular effects of this compound are mediated through its metabolites. Since the tumor suppressor p53 protein responds to cellular stress, and mediates cellular responses to environmental stress and DNA damage, we investigated whether p53 levels were affected in human embryonic lung fibroblasts exposed to butadiene diepoxide, the most genotoxic metabolite of butadiene. We found that under experimental conditions where glyceraldehyde-3-phosphate dehydrogenase (G3PDH) levels remain unchanged after western blot analysis, butadiene diepoxide was effective in elevating p53 levels in exposed fibroblasts at concentrations as low as 1 micromolar. Levels of p53 were elevated 10 fold at 1 micromolar butadiene epoxide concentration, and continued to increase as the concentration of butadiene diepoxide increased. Levels of p53 were maximal when butadiene diepoxide concentrations of 200 micromolar were utilized, and at this point p53 levels were elevated approximately 40 fold as compared to control fibroblasts. Further investigation by pulse chase analysis revealed that p53 stability was mainly responsible for the elevated p53 levels in butadiene diepoxide exposed fibroblasts. Our data also revealed that levels of acetylated p53 at lysine 382 increased in correlation to the elevated p53 levels in butadiene diepoxide exposed cells. Since p53 acetylation has been found to be important in stabilizing and regulating p53 function in response to genotoxic stress in other systems, our results suggest that p53 acetylation at lysine 382 may play a similar role in human fibroblasts exposed to butadiene diepoxide.

1446 TUMOR FORMATION IN MICE BY STYRENE: INDUCTION OF CELL PROLIFERATION IN THE LUNG BY STYRENE METABOLITES.

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Styrene is a high production volume chemical (approx. $2x10^7$ tons/year) used as a monomer for plastic productions. Mice develop lung tumors when exposed chronically to styrene. The relevance of these findings for humans is under investigation. Epidemiological data provide no evidence for a carcinogenic potential in humans. Styrene is metabolised to styreneoxide. This electrophilic metabolite is assumed to cause genotoxicity and consequently lung tumors in mice. Research on the metabolism, kinetics and genotoxic effects of styreneoxide in mice and rats did not disclose a clear-cut model for tumor formation depending predominantly on styreneoxide. Thus styreneoxide cannot be seen as the only ultimate toxicant. Recent mechanistic studies demonstrated that the induction of cell proliferation in the lungs of mice is a major cause of tumor formation by styrene rather than a genotoxic effect. We tested styrene and five of its metabolites (α-phenylethanol, β-phenylethanol, phenylacetaldehyde, acetophenone, phenylacetic acid) for induction of cell proliferation in the lungs of CD-1 mice and Fisher rats. Test substances were administered i.p. The cell proliferation in the lungs was quantified by the S-phase response (BrdU labelling index). It was determined 24 and 72 hours post application in large and medium bronchi, terminal bronchi and alveoli. Highest increases of cell proliferation were observed in the alveoli of mice treated with β-phenylethanol, phenylacetaldehyde and phenylacetic acid, with the aldehyde causing the most pronounced effect. This was observed 24 and 72 hours post application. Interestingly the oxidation at the β-carbon of styrene is an important pathway in mice, but not in rats. This metabolism leads to phenylacetaldehyde and phenylacetic acid, the most potent inducer of cell proliferation in this study. A considerable contribution of these metabolites to the tumor formation by styrene exposure would explain the differences between mice and rats and may provide a better basis for the assessment of risks in humans.

1447 FUNCTIONAL CHARACTERIZATION OF C190T POLYMORPHISM IN HUMAN N-ACETYLTRANSFERASE 2 (NAT2) GENE.

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N-acetyltransferase 2 (NAT2) is an important phase II enzyme, which catalyzes N-acetylation (detoxification) and O-acetylation (activation) of many drugs and environmental carcinogens. Genetic polymorphisms in the NAT2 gene have been associated with differential susceptibility to cancers and drug toxicity from these compounds. Single nucleotide polymorphisms (SNPs) have been identified in the human NAT2 coding region. Their effects on NAT2 expression, stability, and catalytic activity have been investigated in our laboratory. A new allele *NAT2*19* recently was identified. This variant allele possessing the C190T (R64W) SNP was cloned and expressed in yeast (*Schizosaccharomyces pombe*). Substantial reduction in expression of NAT2 immunoreactive protein and protein stability were observed for *NAT2*19* (half-life=2.71 min) when compared with the reference rapid acetylator allele *NAT2*4* (half-life=36.4 min). No significant differences in mRNA expression or transformation efficiency were observed following Northern and Southern blotting. The enzymatic activities for N-acetylation of two arylamine carcinogens (2-aminofluorene, 4-aminobiphenyl), and a sulfonamide drug (sulfamethazine) were over 100-fold lower for NAT2 19 compared to reference NAT 4. In addition, activity for O-acetylation of the heterocyclic amine carcinogen 2-amino-1-methyl-6-phenylimidazo [4, 5-b] pyridine was 24-fold lower. The results show that *NAT2*19* encodes slow acetylator phenotype for both N- and O-acetylation. Our findings define more clearly the effects of individual SNPs on human NAT2 expression, stability and catalytic activity. This work is partially supported by USPHS grant CA34627 from the National Cancer Institute.

1448 A NOVEL REGULATORY MECHANISM FOR TYROSINE HYDROXYLASE.

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Tyrosine Hydroxylase (TH) is regulated by the reversible phosphorylation of serines 8, 19, 31 and 40. The physiological significance of phosphorylation of the serines remains enigmatic, as pan phosphorylation is not obligatory for catecholamine biosynthesis. The purpose of our study was to characterize the physiological relevance of the heterogeneous phosphorylation of TH by identifying novel interacting proteins. Serine 19 is arguably unique due to its requirement of 14-3-3 binding after phosphorylation for optimal enzyme activity, although it has been suggested that phosphorylated serine 40 also binds 14-3-3. 14-3-3 proteins are prevalent in mammalian brain and isoforms of 14-3-3 serve diverse functions including the regulation of intracellular signal transduction cascades by serving as a scaffold between proteins. To characterize proteins that interact with TH after phosphorylation on serine 19, this amino acid was mutated to alanine (THS19A), since it was reported that a serine to alanine mutation promotes a stable interaction between proteins. For the identification of proteins that interact with TH, THS19A was used as the bait in the yeast two-hybrid system and two cDNA clones were identified, consisting of amino acids 147-262 of mouse-derived PITSLRE. PITSLRE is a serine/threonine kinase and characterization of the corresponding human cDNA, in the form of a GST fusion protein, encoding amino acids 1-373 of a splice variant of the a isoform of human PITSLRE demonstrates an interaction between TH and GST-PITSLRE (1-373). Additionally, *in vitro* transcribed and translated TH interacts with full-length FLAG-PITSLRE immunoprecipitated from COS-7 cells. TH does not serve as a substrate for PITSLRE in an *in vitro* kinase assay. However, PITSLRE prevents the interaction between TH and 14-3-3 in HEK 293 cells through a mechanism, which remains to be characterized. It appears that PITSLRE may serve as a negative regulator of TH activity and therefore, possibly catecholamine biosynthesis.

1449 IDENTIFICATION THROUGH GENE EXPRESSION OF A TOXIC RESPONSE IN THE RAT FOR A COMPOUND THAT EXHIBITS OVERT TOXICITY IN HUMANS, BUT NOT IN RATS.

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The classical measures of toxic response have been found to be insensitive to certain known hepatotoxicants, such as those that cause damage in humans, but whose effects are not detectable within the mammalian species used in pre-clinical trials. The implementation of a more sensitive measure of the toxic response to identify this type of compound is needed to assess accurately the potential toxicity of compounds in humans. For a variety of reasons, gene expression measurements from animal models may provide the means necessary to determine the eventual toxic response observed in humans. Tacrine is one such compound where an overt hepatotoxic response is observed in humans, but not in pre-clinical animal models. To identify the genes that characterize a response in the absence of overt hepatotoxicity, tacrine was administered to Sprague-Dawley male rats and liver tissue was isolated at various time points post-exposure. Using the Affymetrix RGU_34 GeneChip® set as a platform for measurement of gene expression levels, we have identified rat genes that are strong indicators of a toxic response for tacrine, but not the vehicle-treated controls. In addition, these genes have been previously shown to be good collective indicators of toxicity using samples extracted from rats treated

with known hepatotoxicants including acetaminophen (APAP), carbon tetrachloride (CCl_4), diclofenac sodium, and alpha-naphthylisothiocyanate (ANIT). These results may indicate that marker genes in the liver detected by administration of known toxicants are more sensitive for detection of the toxic response than classical methods. This expression information can also be used to identify toxic compounds that would otherwise be missed as part of the pre-clinical testing process.

1450 CHROMIUM-INDUCED CHANGES IN GENE EXPRESSION IN HEPG2 CELLS, AND IN WINTER FLOUNDER AND MICE LIVER USING MACRO- AND MICROARRAYS.

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Chromium is the dominant toxicant found at several Superfund sites around the United States, including sites in Odessa, TX and a estuarine sites in Maine and South Carolina. However, chromium's effects are not well understood. Therefore, we have attempted to characterize the effects of Cr(III) and Cr(VI) on gene expression in HepG2 cells, as well as male winter flounder *(Pseudopleurinectes americana)*, and male CD-1 mice. HepG2 cells were exposed to Cr(VI), as chromium oxide and Cr(III), as chromium chloride hexahydrate to determine their toxicity. Cr(VI) caused death of 80% of the HepG2 cells at 1μM, while Cr(III) did not cause cell death, but significantly decreased growth at 100μM. Therefore, cells were treated with 20μM Cr(III), RNA was extracted and an array was incubated and analyzed. Several genes showed altered expression due to Cr(III), including α-1 antichymotrypsin, cathepsin D, and C-1. RT-PCR was performed to confirm differential expression of several of these genes. Additionally, mice and winter flounder were examined as *in vivo* models. Winter Flounder was injected with Cr(III) and Cr(VI) at 400 and 25μg/kg, respectively, and euthanized 24 hours later. mRNA was extracted from liver and subtractive hybridization was performed to enhance the production of differentially expressed clones, that were amplified by PCR and used to produce macroarrays. The macroarrays will be used to confirm differential expression, and clones demonstrating altered expression will be sequenced. Additionally, mice were treated with Cr(III) in the water for 14 days at 1000 and 2000 ppm, which approximates 7.5-15mg/kg/day. Superoxide dismutase was increased in treated mice, suggesting Cr(III) caused oxidative stress. Microarrays will also be performed on mRNA extracted from mouse livers to determine if similar genes are induced in mice, humans and winter flounder by Cr(III).

1451 DEVELOPMENT OF A NOVEL METHOD FOR ANALYSIS OF TRANSCRIPTIONAL CHANGES IN TRANSITIONAL EPITHELIUM FROM URINARY BLADDERS OF RATS EXPOSED TO DRINKING WATER DISINFECTION BY-PRODUCTS.

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Epidemiologic studies in human populations drinking disinfected water have consistently shown an increase in the risk of urinary bladder cancer. Increased incidence of transitional cell carcinoma of the urinary bladder is associated with consumption of chlorinated surface waters. The transitional cell epithelium is therefore the target for chemically-induced toxicity and carcinogenicity in the urinary bladder. After chronic treatment to MX alone or a mixture of DBP's, transitional epithelial cells had marked anisokaryosis and megalokaryocytes associated with focal hyperplasia. To detect and analyze biologic events which contribute to transitional cell carcinoma, we developed a method by which only the target cell population was removed from the urinary bladder of male rats and the RNA isolated for transcriptional analysis using the Clontech™ Rat Atlas 1.2. Twelve male Long-Evans rats, 4 per group, were exposed to a mixture of potassium bromate, MX, chloroform, and bromodichloromethane administered in drinking water in a mixture of low doses of 0.02, 0.005, 0.4 and 0.07 g/L, or as a mixture of high doses of 0.4, 0.07, 1.8 and 0.7 g/L, respectively. In the present study, animals were exposed to deionized water or the mixture solutions for 3 weeks, then euthanized and necropsied. At necropsy the urinary bladder was instilled with 500 microliters of Trizol™ reagent, set for 7 minutes, then flushed to remove the lining cells. The amount of total RNA extracted per bladder after *in situ* Trizol treatment ranged from 12 to 363 micrograms. Total RNA was used to perform the cDNA array analysis which was then verified using real-time PCR, (TaqMan™). Ths method will allow study of the biology of the transitional epithelium of the urinary bladder and detection of altered gene expression for improved understanding and defining of biologically relevant responses to urinary bladder carcinogens. This abstract does not reflect EPA policy.

1452 ALTERATIONS IN GLOBAL GENE EXPRESSION INDUCED BY AFLATOXIN B_1 IN YEAST EXPRESSING HUMAN CYTOCHROME P450 1A2.

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Aflatoxin B1 (AFB) is a human hepatotoxin and hepatocarcinogen produced by the mold *Aspergillus flavus*. In human, AFB is bioactivated by cytochrome P450 (CYP) enzymes, mostly CYP1A2, to genotoxic epoxide which forms N^7-guanine DNA adducts. To characterize the transcriptional responses to AFB genotoxic insult, *S. cerevisiae* strain YHE2, engineered to express human CYP1A2 or vector, was grown to a density of 5 X 10^6 cells/ml and exposed to AFB (10, 25, 50, 100 μM) or vehicle control (DMSO) for 2 or 4 hours in ura(-) medium at 30°C. AFB did not result in significant cell death at 2 hr, determined by colony forming ability, but did induce dose and time-related increases in mitotic recombination measured by *Trp* reversion assay. No cell death or mitotic recombination was observed in the vector expressing strain. cDNA was synthesized from total RNA isolated from treated and control CYP1A2-expressing cells using oligo $d(T)_{15}$ primers and aminoallyl-dUTP, which was then chemically coupled with Cy5 or Cy3 fluorescent dye, respectively. The labeled cDNA pools were admixed and hybridized to a yeast microarray spotted with 6, 246 cDNAs, representing the entire yeast genome. Approximately 200 transcripts were changed by at least two-fold with respect to time and/or dose, which included stress response, protein degradation, DNA replication, RNA synthesis and cell cycle regulation genes, as well as approximately 60% with unknown identity and/or function. Expression of 12 genes increased consistently in a time and/or dose-dependent fashion. Decreased expression was observed for about 30 genes at all conditions except the highest dose (100 μM), at which over 150 genes decreased. Cluster analysis suggested a role for the recombination-mediated DNA repair mechanism, as Rad51 and ribonucleotide reductase genes (RNR1, RNR2, RNR3, RNR4) were co-upregulated in a dose- and time-dependent manner. (Supported by NIH grants ES07033 and ES05780).

1453 ALTERED LIVER GENE EXPRESSION AND POST-TRANSLATIONAL PROTEIN MODIFICATIONS IN RESPONSE TO LETHAL OR NON-LETHAL *LISTERIA MONOCYTOGENES* INFECTION IN FEMALE BALB/C MICE.

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Infection of mice with *L.monocytogenes* (*LM*) is widely-used to study host response to intracellular bacterial pathogens and to examine immunotoxic effects of therapeutics. *LM* is also a food-borne pathogen and is thought to act in part by modifying host cell signal transduction and utilization of the host's actin-based cytoskeleton. While this model is well studied, effects on murine gene expression remain largely unknown. The objective of this study was to determine if infection induced differential gene expression or protein modification in the liver, a primary target organ. Mice received by i.v. route either vehicle, $1x10^3$ (non-lethal), or $5x10^4$ CFU *LM* (lethal) and were sacrificed 1, 2, 4, and 14 days later. Levels of RNA were measured with Affymetrix Genechip® arrays. Differentially phosphorylated proteins were identified using gel separation and MALDI-TOF. Infectious burden, clinical and histopathology confirmed infection and showed significant effects in the high dose group. Multiple genes were found to be differentially expressed in a reproducible and dose-related manner. Classes of genes altered included immune-function genes (lymphocyte antigen 6 complex, macrophage expressed gene 1), genes associated with liver toxicity (Ntcp, hypoxia inducible factor1, HSP 70), and genes involved in cell signaling (EGF-R, IGFBP-4, IGFR-2). In addition, using data analysis and modeling tools we have identified a preliminary set of genes that may be useful in assessing if an infection with *LM* will be lethal or non-lethal. Protein analyses indicate that, γ-actin, ATP-synthase β-chain and CDC5 kinase regulatory subunit1 undergo differential tyrosine phosphorylation. These results indicate large-scale differences in gene expression and patterns as well as in protein modification in livers of exposed mice. Supported in part by NIAID Contract N01-AI-05417

1454 TRANSPLACENTAL EXPOSURE TO ESTROGENS INDUCES A DISTINCTIVE GENE EXPRESSION PROFILE IN THE FETAL UTERUS OF THE RAT.

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In order to understand the molecular events associated with the estrogenicity of different chemicals, we have used microarray technology to determine whether there is a common set of genes which expression profile could be traced to estrogenic com-

pounds exposure during organogenesis and development. Changes in patterns of gene expression were determined in the developing uterus and ovaries of Sprague-Dawley rats on gestational day (GD) 20, exposed to different doses (s.c.) of 17 α-ethynyl estradiol (0.5, 1, and 10 μg EE/kg/day); bisphenol A (5, 50, and 400 mg BPA/kg/day) and genistein (0.1, 10 and 100 mg/kg/day) from GD11 to GD20. Gene expression was evaluated using high density oligonucleotide arrays. A dose-dependent analysis of the gene expression profile revealed that each estrogenic compound induces a distinctive transcript profile, reflecting their estrogenicity as well as their molecular mechanism of action. Of the ~7000 annotated rat genes and 1740 ESTs, analyzed in this study, there were 366, 398 and 344 genes whose expression level shows some evidence of treatment effect (up or down regulated) by EE, BPA and genistein, respectively, relative to the vehicle control. From those genes, the expression of 66 was consistently and significantly regulated in the same direction, although at different magnitudes, by the three estrogenic compounds. Among the genes regulated by estrogens there are some that have been described in the literature including intestinal calcium-binding protein, progesterone receptor, 11-β-hydroxylsteroid dehydrogenase type 2, interleukin 4 receptor, and insulin-like growth factor 1. However, most of the genes responsive to estrogen exposure had not been previously identified. These data demonstrate that transplacental exposure to estrogens changes the gene expression profile of estrogen-sensitive tissues, and that the uniqueness of these transcript profiles could be a valuable tool to determine the estrogenicity of different compounds.

1455 EFFECTS OF GESTATIONAL AND LACTATIONAL EXPOSURE TO DIETHYLSTILBESTROL ON TESTICULAR GENE EXPRESSION USING cDNA MICROARRAYS AND REAL-TIME PCR.

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To explore the molecular events underlying the adverse effects on sperm quality following developmental exposure to estrogenic chemicals, we have constructed cDNA microarrays to examine testicular gene expression in B6D2F1 offspring of mice exposed by gavage to 10 μg/kg diethylstilbestrol (DES) from gestational day 12 to postnatal day 21. Replicate gene expression profiles were examined in male offspring at 3, 15 and 45 weeks of age to determine if the effects on gene expression paralleled the long term decreases in testis weight, sperm count, and sperm fertilizing ability, as previously described. A mouse cDNA microarray containing approximately 1948 genes was constructed and used to compare testicular gene expression in the offspring of DES-exposed mice to that of vehicle-exposed mice. An independent reference design coupled with paired t-tests was used to identify genes significantly ($p<0.05$) altered in expression by DES. Based on adjusted p values, there was only one gene significantly altered at 3 weeks of age (a component of the 20S proteasome). At 15 weeks of age, there were 46 genes significantly altered in expression. There were no significant changes at 45 weeks of age. Raw p values and functional annotation were used to prioritize the selection of genes for verification. Using real-time PCR, both age and dose-dependent changes in gene expression were examined. There was a significant decrease in the expression of ER alpha mRNA at 3 weeks of age ($p<0.01$), while mRNA expression was below the limits of detection in 15 and 45 week old mice. Other genes selected for verification include inhibin, the orphan receptor TR2, and Xmr, a component of the synaptonemal complex. These results suggest that the adverse effects on sperm fertilizing ability may be due to altered expression of ER alpha, and possibly other genes, in the testis.

1456 THE EFFECT OF GLUTATHIONE DEPLETION ON GENE EXPRESSION IN HEPG2 CELLS.

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Glutathione is known to play a critical role in the control of cellular homeostasis through reductive and anti-oxidant action and through the regulation of specific intracellular cell signalling. We wished to determine the influence of glutathione depletion on the expression of a range of stress-response genes in cultured human HepG2 cells. Following treatment of cells with buthionine sulphoximine (1.0mM) for 6 and 24h, the concentration of reduced glutathione (GSH) was depleted to 60 ± 3.1 and 17 ± 1.5 % of control values respectively (n = 5). There was no associated increase in oxidised glutathione or evidence of cytotoxicity as measured by intracellular ATP concentrations and lactate dehydrogenase leakage. Differentially expressed genes were identified by suppressive subtractive hybridisation (SSH) and through the use of "Phase 1" human microarrays customised for toxicity detection. A wide range of genes known to be responsive to oxidative stress showed no change in expression under these conditions. However, consistent increases (1.5 - 2.8 fold with correlation coefficients > 0.95 in the microarray analyses) in sub-sets of genes (approximately 50)were indicative of apparent adaptive responses. A total of 26 genes were also down-regulated following microarray analysis. These include transferrin and UDP glucuronosyltransferase 2B. In particular, induced genes included c-jun in accord with previous reports of AP1 activation by GSH depletion. There was an increase in glutathione peroxidase and ubiquitin (by SSH) that may reflect adaptation. Eleven cytochrome P450 (CYP) forms were also over expressed which suggests an inter-relationship between control of GSH homeostasis and CYP regulation.

1457 MICROVESICULAR STEATOSIS INDUCED BY SHORT CHAIN CARBOXYLIC ACID ANALOGS: EVALUATION OF GENE EXPRESSION AND CORRELATION TO MITOCHONDRIAL TOXICITY.

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Hepatotoxicity characterized by microvesicular steatosis features abnormal accumulation of numerous small cytoplasmic lipid droplets in hepatocytes. Fulminant or progressive cases may lead to liver failure and death. Experimentally, short-chain fatty acids are known to induce hepatocellular microvesicular steatosis. The precise mechanism(s) whereby short-chain fatty acids induce microvesicular steatosis are unknown, although there is evidence implicating disruption of mitochondrial function. The identification of gene changes that correlate with microvesicular steatosis concomitant with biochemical indices would provide a better understanding of how the toxicity occurs. To this end, a series of short chain fatty acids including valproic acid (VPA), cyclopropane carboxylic acid (CPCA), 4-pentenoic acid (4PE), pivalic acid (PIV), butyric acid (BTR) and propionic acid (PRO) were tested *in vitro* for their ability to inhibit mitochondrial beta oxidation and *in vivo* for 1, 3 and 7 days to evaluate their ability to induce microvesicular steatosis in rats. VPA, CPCA and PIV resulted in microvesicular steatosis 1-3 days post treatment while PRO and BTR did not. In agreement with this finding these compounds inhibited mitochondrial beta-oxidation in a dose dependent manner *in vitro* and caused a reduction in mitochondrial respiration, whereas no inhibition was evident with PRO or BTR. Gene expression results showed a regulation in genes involved in beta-oxidation, including thiolase, acyl-coa hydrolase and cytochrome P450 2C11, as well as other genes not previously associated with microvesicular steatosis. Overall, these results support altered hepatic mitochondrial function as a causative mechanism of the toxicity induced by short-chain fatty acids and provide potential markers for this toxicity.

1458 CONCORDANCE OF DEGREE OF RENAL INJURY WITH GENE EXPRESSION IN INDIVIDUAL ANIMALS TREATED WITH THE NEPHROTOXICANT CISPLATIN.

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As part of a coordinated evaluation of the application of genomic methodologies to mechanism-based risk assessment, the Nephrotoxicity subteam of the ILSI Genomics and Proteomics Subcommittee examined the relationship between traditional toxicologic endpoints and gene expression responses to prototypic nephrotoxic drugs in Sprague-Dawley rats. As part of one study, 5 male rats received a single ip injection of 5 mg/kg cisplatin. Six days later, the animals were sacrificed, standard serum chemistry and urinalysis parameters were measured, and the kidneys were processed for histopathology and RNA. Gene expression profiles were evaluated with the 700 gene Phase-1 Rat(CT) microarray on samples from individual animals, pooled controls, and pooled treated animals. 3 of the 5 animals had moderate tubular necrosis and regeneration, and highly elevated serum levels of BUN and creatinine. One animal had mild tubular necrosis and regeneration, and no evidence of kidney injury was observed in a fifth rat. Both of these latter animals had normal BUN and creatinine levels. The gene expression induction profiles were highly correlative among the 3 animals that had the same degree of renal damage and altered serum chemistry, and with the pooled sample from treated rats. In a subset of genes, there was concordance between the magnitude of the induction and the type and extent of tubular injury seen. The observed gene induction profile was highly concordant with results from pooled animals in an earlier, independent study using the same dose of cisplatin. Genes significantly up-regulated in animals with evidence of renal toxicity included damage-inducible genes like GADD153, clusterin, and cyclin G. Renal tubular markers like OAT3, HNF4, and EGF were

significantly down-regulated in these animals. The observed patterns of gene induction in this study were consistent with known mechanisms of cisplatin nephrotoxicity, highly reproducible, and reflective of the extent of damage observed in histopathologic analysis.

1459 TEMPORAL EXPRESSION PATTERNS OF GENES IN THE UTERI OF IMMATURE, OVARIECTOMIZED MICE TREATED WITH ETHYNYL ESTRADIOL.

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Estrogens are classically defined as compounds that are capable of inducing a trophic effect on the uterus. This physiological change has been linked to alterations in the expression of estrogen-responsive genes, but the cascade of molecular events remains unknown. In the present study, the effects of ethynyl estradiol (EE), a synthetic estrogen, on global gene expression in then uterus were examined in immature, ovariectomized C57BL/6 mice. Mice were gavaged with 0.1mg/kg EE or vehicle, and uteri harvested at 0, 2, 8, 12, or 24 hr. Overall, while no differences in uterine weight were found between treatment groups (p=0.296) from 0 to 24 hrs, changes in global gene expression levels were observed during this period. Mu11KsubA Affymetrix GeneChips were used to assess relative expression levels of over 6000 genes in uterine tissue of two animals from each treatment and time-matched vehicle treated (control) group. Data were then screened using standardized t scores to identify gene expression changes outside the normal range of variation for the control group. Treated values were standardized using i) a global control mean and standard deviation assuming no time effect among the control observations, or ii) the average and standard deviation of the time-matched control values. These methods were found to give different results, with the time-matched control approach resulting in the identification of fewer active genes. Large t scores were interpreted to indicate active gene responses. Overall the 24 hr time point had the largest t scores as determined by average ranks and summary statistics over time. The nature of the changes over time was characterized using a parametric model based on the general shape of the time-effect relationship.

1460 EVALUATION OF DIFFERENTIAL GENE EXPRESSION IN THE SPLEENS OF RATS AT SEVERAL TIMEPOINTS AFTER INJECTION OF CYCLOPHOSPHAMIDE.

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The toxicity of drugs and chemicals is dynamic with overlapping stages of drug metabolism, direct and indirect cellular damage, and repair. Recognizing this complexity in the toxicity response, Phase-1 evaluates gene expression by sacrificing rats at 6, 24 and 72 hours after a single intraperitoneal injection of a compound at two concentrations. For this study Sprague-Dawley rats were dosed with several immunotoxicants. Cyclophosphamide dosed at 25 and 100mg/kg induced lymphoid depletion in the spleen at 72 hours. The spleen of each rat was harvested and total RNA was isolated for transcription to cDNA with dual cy-3, cy-5 labeling and hybridized on the PHASE-1 rat 700 gene toxicology array. The gene expression levels were compared across time points and across doses. The differential gene expression with cyclophosphamide was also compared with other immunotoxicants. The ability to detect toxicity by determining differential gene expression improves the process of evaluating toxicity in pre-clinical studies with rats

1461 THEOPHYLLIN INDUCED DIFFERENTIAL GENE EXPRESSION IN THE HEART.

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Gene expression data generated using PHASE-1's comprehensive toxicological, 700-gene micro array was evaluated in order to compare gene expression profiles for different cardiac toxicants. Labeled cDNA was produced by reverse transcription of mRNA isolated from tissue harvested at 6, 24, and 72 hours after rats received a single intraperitoneal injection of a cardiac toxicant at two concentrations. This cDNA was then hybridized onto arrays composed of genes empirically determined to be toxicologically relevant. The gene expression showed correlations between theophyllin (cyclic phosphodiesterase inhibitor) dosed at 25 and 100 mg/kg, and other cardiac toxicants, including nifedipine (calcium channel blocker) dosed at 30 and 120 mg/kg. The data corresponded to the histologic evidence of myocardial degeneration and inflammation observed at 72 hours and to the compound's mechanism of toxicity. This study demonstrates how microarray technology used at PHASE-1 can be a powerful toxicological tool.

1462 SEXUAL DIMORPHISM OF HEPATIC GENES EXPRESSED IN HEXACHLOROBENZENE (HCB)-TREATED RATS.

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HCB is an epigenetic carcinogen that predisposes females to the formation of liver tumours. Short-term exposure (5 days) to HCB promotes, with greater efficiency in females than in males, rat liver tumours induced by DEN injection 95 days following the last treatment. The objective of this study was to determine gender-specific differences in hepatic gene expression and their relationship to tumour promotion. To address this objective four experimental groups of rats were used: 1. vehicle-exposed males (control); 2. vehicle-exposed females (controls); 3. HCB-treated males; 4. HCB-treated females. Rats were administered corn oil (vehicle) or HCB (100 mg/kg) by gavage for 5 consecutive days and sampled on day 50 of the experiment. Cellular mRNA was isolated from the liver of each rat (n=4/group) and used to screen an Atlas array 1.2 II (Clontech) containing 1176 genes. Results indicate that in control male rat liver, 137 genes could be detected using this array, with 94% of expressed hepatic genes being common to each individual. In the female livers, 186 genes were expressed and 82% of these were common to each individual. These results indicate that, of the genes screened, there are 26% more genes expressed in female livers than in males and that there is more variability in the expression of these genes between individual females than between males. In HCB-treated males, there were 161 genes expressed, i.e. an 18% increase in the number of genes expressed as compared to controls, but only 71% of these were expressed in all four males. In HCB-treated females, 153 genes were expressed, i.e. an 18% decrease in the number of genes expressed as compared to controls, and 83% of these were found in each individual female. Male *versus* female differential comparisons of HCB-modulated genes will be discussed in relation to gene clusters that may be responsible for the gender-specific response in liver tumour promotion. (Supported by TSRI, Health Canada)

1463 QUANTITATIVE PROFILE OF TESTICULAR GENE EXPRESSION PATTERNS IN MICE EXPOSED TO DIETHYLSTILBESTROL DURING GESTATION AND LACTATION .

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Previously, we have reported decreases in sperm fertilizing ability in male offspring exposed to diethylstilbestrol (DES) during gestation and lactation. In this study, a mouse cDNA microarray containing approximately 1948 genes was used to profile the testicular gene expression of the offspring. Pregnant C57BL/6 female mice bred with DBA/2 males were gavaged with 10 ug of DES/kg of B.W/day from gestational day 12 to postnatal day 21. The construction of cDNA microarray and gene expression identified by computing t-statistics and P-values have been reported in a separate presentation. In this report, the fluorescence measurements were analyzed using two interconnected mixed models, a ¡°normalization" model and a ¡°gene¡± model. The normalization model accounted for experiment-wide systematic effects that could bias inferences made on the data from individual genes. The residuals from this model represent ¡°normalized¡± values, and were the input for the gene model. The gene model was fitted separately to the normalized data for each gene, allowing inferences to be made using separate estimates of variability. Differences between Least Squares Means for treatment groups were estimated using Scheffe adjustments. At 15 weeks of age, the expression of 20 genes in the exposed males were four times or higher of the levels in the controls. Additional 39 genes were expressed at least twice of that in the controls. Among the genes which expression was suppressed, 31 genes were expressed less than one fourth of that in the controls and another 31 were expressed at less than half of the control levels. Over expression of prolactin receptor, serine/threonine kinase 10, and tyrosine phosphatase and under expression of prostaglandin E receptor 1 are among the several prominent changes observed in the testes of DES exposed offspring.

1464 IDENTIFICATION OF DIFFERENTIAL EFFECTS OF BENZO(A)PYRENE AND 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN ON GENE EXPRESSION IN HUMAN PLACENTAL JEG-3 CELLS USING CELL CYCLE AND DNA MICROARRAY ANALYSES.

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Maternal cigarette smoking disrupts placental growth and function, and the aryl hydrocarbon compound benzo(a)pyrene (BaP), a major toxicant in cigarette smoke, has been shown to inhibit proliferation and growth factor expression in cul-

tured human placental cells and JEG-3 choriocarcinoma cells. This study further investigated the effects of BaP on cell cycle and gene expression in JEG-3 cells in comparison with 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), the reference ligand for the aryl hydrocarbon receptor (AhR), and benzo(e)pyrene (BeP), a weak ligand for the AhR. JEG-3 cells treated with 10 uM BaP for 48 hours showed a 75% decrease in [3H]thymidine incorporation, whereas 10 nM TCDD had no effect. Flow cytometry analysis showed that BaP induced a G2M cell cycle arrest, while TCDD and 50 uM BeP had no effect on cell cycle phase distribution. Cell cycle regulatory proteins were analyzed by Western immunoblot and immunohistochemistry. BaP markedly induced expression of nuclear p21 protein, a major inhibitor of cyclin-dependent kinases. Although BaP had no effect on total cellular p53 levels, phosphorylation of p53 at serine 15 was markedly increased. In contrast, neither TCDD nor BeP had any effect on the expression of p21 or p53 phosphorylation. Lastly, a human cancer cDNA microarray was used to evaluate changes in gene expression induced by treatment with BaP and TCDD. Data were analyzed using Atlas Image, Cluster and Treeview programs. BaP down-regulated 7 genes, while 3 were up-regulated. In comparison, TCDD was found to down-regulate 9 genes, while 1 was up-regulated. Of these, BaP and TCDD had similar effects on only 3 genes. In summary, although BaP and TCDD are both ligands for the Ah receptor, only BaP produces G2/M cell cycle arrest with induction of p21 and p53 serine phosphorylation that is characterized by a unique pattern of gene expression in JEG-3 cells. (Supported by NIH ES07375)

1465 DIFFERENTIALLY EXPRESSED SEQUENCES IN THE MUMMICHOG AS A RESULT OF ANTHRACENE EXPOSURE.

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Approximately 40% of all USEPA Superfund sites have some sort of polycyclic aromatic hydrocarbon (PAH) contamination. Many of these sites are scheduled for remediation and tools are needed to assess whether efforts have been successful. Knowledge of the induction or suppression of specific genes as a result of exposure may be useful in understanding the mechanisms of PAH toxicity. We are attempting to generate a fingerprint of exposure to the PAH anthracene using the differential-display reverse transcription polymerase chain reaction (DD RT-PCR) technique. This technique has produced a series of cDNA fragments that will be used in constructing a microarray specific to anthracene. We then compare these fragments that have been detected to sequences of known genes in order to identify possible physiological consequences of exposure to anthracene. Mummichogs (*Fundulus heteroclitus*) are a non-migratory estuarine fish used to model exposure because they have contact with contaminated sediments over the full course of their lifetime. The fish were subjected to 7-day static renewal tests using environmentally relevant concentrations of 0 ppb, 27 ppb, 50 ppb, and 80 ppb of anthracene. The fish were euthanized, livers were removed, and total RNA was extracted. The mRNA was reverse transcribed into cDNA, amplified by rationally designed primers and run on a differential display gel. Sixteen cDNA fragments that represented differentially expressed mRNA sequences were isolated, amplified, and sequenced. The GENBANK database was searched to compare these mummichog sequences to gene sequences of known function. Three sequences thus far have shown similarity to known genes—CYP2N2 in the mummichog, insulin-like growth factor I in the Japanese flounder (*Paralichthys olivaceus*), and trypsinogen in both the Atlantic cod (*Gadus morhua*) and the Maori cod (*Paranotothenia magellanica*). Future work will relate the up- or down-regulation of these genes to the physiological impacts of anthracene exposure.

1466 THE LONG-TERM EFFECTS OF NEONATAL EXPOSURE TO ENDOCRINE DISRUPTORS ON TESTICULAR GENE EXPRESSION IN MICE.

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In order to evaluate the feasibility of cDNA microarrays for the risk assessment of endocrine disruptors (EDs), alteration of gene expression profiles was analyzed in adult mouse testes after neonatal exposure to diethylstilbestrol (DES), bisphenol A (BPA) or genistein (Gen), using cDNA microarrays. Analysis with Mouse GEM I revealed that expression levels of 34, 38 and 12 genes were changed in testes of DES-, Gen- and BPA-treated 12-week-old mice, respectively. Gene expression profiles were very similar between DES- and Gen-treated mice, but that of BPA-treated mice was quite different. These results suggest that gene expression profiles might be feasible for grouping of EDs in terms of their effects. Further, it is suggested that mechanisms of BPA action may be different from those of DES and Gen, although they are all estrogen-like compounds. Next, we investigated transition of the expression profiles in testes of DES-treated mice during 4-12 weeks of age using in-house cDNA microarrays. Many of genes affected by DES were up- or down-regulated at restricted periods, but some genes were continuously up-regulated or shifted from down-regulated state to up-regulated state. These results suggest that the transition patterns may represent the gene expression cascades that were affected by EDs. Moreover, the gene that was up-regulated continuously in the long term might be a candidate for the biomarker indicating that the exposure of individuals to EDs occurred at some sensitive period. We propose that cDNA microarray is a useful tool, which provides us a bird's eye view of global effects of EDs on the gene expression.

1467 EFFECTS OF DIETARY EXPOSURE TO GENISTEIN AND METHOXYCHLOR ON THE GENE EXPRESSION PROFILE OF THE MAMMARY GLAND IN MALE RATS.

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Our previous study has shown that the isoflavone genistein (GE) altered the toxicological effect of the pesticide methoxychlor (MXC) on the growth pattern of mammary glands in male rats. The objective of this study was to determine the gene expression profile in the mammary glands of GE- and MXC-treated male rats. Sprague-Dawley rats were fed a soy- and alfalfa-free diet containing GE (800 ppm) and MXC (800 ppm), either alone or in combination throughout pregnancy and lactation. Dietary exposure of the offspring was continued until postnatal day 90, when the pups were killed. Total RNA was isolated from one inguinal mammary gland of each non-littermate (n = 3). Radiolabeled cDNA was generated using Clontech CDS primer labeling and hybridized to Clontech Rat 1.2 arrays (with 1, 176 gene probes). Phosphoimager-detected radio signals were background-adjusted (Atlas Image, Clontech) and analyzed for gene expression patterns (GeneSpring, Silicon Genetics). Both the GE and MXC treatments altered the gene expression profile of the mammary glands. More genes were affected (at least three-fold up- or down-regulation) in the treated groups than in the control group when data values were normalized to the 50th percentile of all the measurements and then to each gene itself. The combination of GE and MXC showed a more prominent effect than either compound alone. Some subsets of genes were only affected in the GE group, only in the MXC group, and only in the combination group (47, 47, and 172 genes, respectively), while 78 genes were affected in all treatment groups. The general pattern of the changes is in close agreement with treatment effects observed at the morphological and biochemical levels. While the toxicological significance of such changes in gene profiles is not yet clear, we expect that further analysis of the data will help guide our research on elucidation of the pathways leading to the GE- and MXC-induced mammary gland effects.

1468 THE USE OF DNA MICROARRAY TECHNOLOGY TO ANALYZE THE EFFECT OF 3, 4-DICHLOROPROPIONANILIDE (DCPA) ON HUMAN T LYMPHOCYTES.

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DCPA or propanil is the active ingredient in post-emergence herbicides such as WHAM! EZ®and is used extensively in the cultivation of rice. Previous work in our laboratory using both an *in vivo* mouse model and an *in vitro* exposure model has demonstrated that DCPA inhibits several important immune system functions including both T-independent and T-dependent antibody responses, cytokine production and natural killer (NK) cell function. Additional studies using the human T lymphoma cell line Jurkat have demonstrated that exposure to 100 µM DCPA decreased the secretion of IL-2. The decrease in IL-2 production is due in part to a decrease in the DNA binding activity of the transcription factors AP-1, NFAT and NF-kB. To understand the overall effect DCPA has on human T lymphocytes DNA microarray technology was employed. Jurkat cells were stimulated with PMA and the calcium ionophore A23187 and exposed to 100 µM propanil or ethanol (vehicle control). The experiment was designed to address three questions: 1) what are the initial or early (15 and 30 m) effects of DCPA exposure? 2) What are the downstream (2 and 8 h) effects? 3) What are the long-term (24 and 36 h) effects? Using the ToxChip created by the NIEHS Microarray Center, we analyzed the mRNA expression patterns of approximately 2000 genes. Results from two independent experiments demonstrated that 92 genes or 4.6% of the genes analyzed were affected by DCPA exposure in each experiment. Some of the effected genes included transcription factors, cell cycle control genes, kinases, transcription/translation genes and phosphatases. Consistent with our previous data, many of the genes whose mRNA expression was decreased in DCPA treated cell are known to be regulated by at least one of the three transcription factors AP-1, NFAT and NF-κB which have decreased DNA binding activity in DCPA treated cells. Supported by NIEHS ES07512

1469 MICROARRAY ANALYSIS OF GLOBAL GENE REGULATION BY TUMOR NECROSIS FACTOR ALPHA.

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Tumor necrosis factor alpha (TNF-α) is an important cytokine with pleitropic effects on cellular functions. TNF-α can mediate cellular responses either in a paracrine or autocrine manner by activating the signaling pathways involved in the expression of appropriate genes. It is well documented in the scientific literature that under certain *in vitro* conditions, TNF-α can quickly and markedly induce the expression of early response genes. In order to investigate the rapid effects of TNF-α we exposed a macrophage cell line, RAW 264.7 cells, for 1 hour to exogenous TNF-α. In two identical experiments we identified 147 cDNAs that were consistently induced > 2 fold. Many of the induced genes fell into 3 categories: cytokine and immune function (TNF-α, macrophage colony stimulating factor, small inducible cytokine A2, immunoresponsive gene 1, IL-15 receptor, CD 48 antigen, TNF α-stimulated ABC protein); cell signaling and transcriptional regulators (p38δ, zinc finger like protein 36 (*zpf36*), c-myc oncogene, toll like receptor 2, guanidine nucleotide binding protein alpha, serum-inducible kinase, fos-like antigen 2), and lipid metabolism (very long fatty acyl coA synthetase and cytochrome p450A10). Interestingly, amongst the 147 consensus genes induced by TNF-α, the most highly induced were TNF-α itself (25 fold) and *zpf36* (12 fold). *Zpf36* is a transcriptional factor known to modify the stability of TNF-α mRNA. Overall, the genes induced are consistent with a known physiological role of TNF-α in amplifying the immunomodulatory response. Currently, studies are ongoing to better understand and tease out the specific TNF-α signaling pathways involved in autocrine regulation of macrophage function.

1470 IDENTIFICATION AND CHARACTERIZATION OF GENES RESPONSIVE TO PERFLUOROOCTANE SULFONIC ACID EXPOSURE USING DIFFERENTIAL DISPLAY AND GENE CHIPS.

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Differential display, first introduced by Liang and Pardee (1992), is a useful method to identify differences in gene expression at the whole genome level. Affymetrix rat genome U34A chip, containing ~8000 full-length annotated genes and EST clusters, has been proven to be a powerful tool in toxicology studies using rat as model system. Perfluorooctane sulfonic acid (PFOS) is the end metabolite of a number of perfluorinated fatty acids analogues extensively used in industrial materials and commercial applications. Few studies have been conducted on this novel compound, and its mechanism of action still remains unclear. In the current study, the restriction fragment differential display (RFDD-PCR) a modified version of the original method and Affymetrix gene chips were used to identify alterations in gene expression levels due to PFOS exposure *in vitro* and *in vivo*. H4IIE rat liver hepatoma cells were treated with solvent control, PFOS at 2ppm and 50ppm in medium for 96hrs. SD rats were oral gavaged with vehicle or PFOS at 5mg/kg/day for either 3 days or 3 weeks. RNA samples were extracted from cells and rat livers and prepared for subsequent analysis. Following the RFDD-PCR procedure, 90 bands on sequencing gel were identified as different among treatment groups. All these candidate genes were sub-cloned and sequenced. Gene chip analysis was conducted by hybridizing U34A chips with experimental samples, and analyzed using microarray suite software and data mining tools. Genes that were differentially expressed in response to PFOS exposure can be clustered as genes coding for fatty acid metabolizing enzymes, nuclear binding factors, and proteins involved in signal transduction pathways. Consistent results were obtained from replicates in gene chips, however, expression profiles *in vitro* and *in vivo* showed only limited similarity.

1471 THE HYPOXIA INDUCIBLE FACTOR, HIF1α, IS CRITICAL TO METAL INDUCED TOXICITY.

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Metal induced toxicity is one of the most important environmental health issues facing humans. Metals, such as nickel, cobalt, lead and cadmium are known or suspected carcinogens. The mechanism of action for the toxicity of these metals remains largely unknown. The process has been linked to a number of factors, including, stress proteins, reactive oxygen species, DNA methylation and others. To identify target genes for metal induced toxicity we utilized DNA microarrays representing approximately 9000 human genes. Hep3B cells were treated with nickel (100 μM), cobalt (100 μM) or hypoxia (1% O_2) for 24 hours. Hypoxia treatments lead to significant expression changes in 61 genes. These genes included known hypoxia regulated genes (e. g. vascular endothelial growth factor and adrenomedullin) and several new hypoxia regulated targets (e.g. Ras suppressor protein and the WSB-1 signaling protein). Greater then 50% of these hypoxia regulated genes were also regulated by nickel and/or cobalt. The overlap between these expression profiles suggests a common signaling system. The most likely target for this central mediator is the hypoxia inducible factors (HIFs). To test the hypothesis mouse embryonic fibroblast (MEFs) from wild type (wt) and Hif1α null (Hif1α-/-) animals were prepared. These two cell types were treated with various concentrations of cobalt and their growth properties were analyzed. In wt MEFs, cobalt exposure (100 μM $CoCl_2$) displayed significant toxicity. Growth rates were decreased by 95% when compared to the untreated controls. The Hif1a-/- MEFs, however, showed little if any toxicity at this concentration. To find the potential targets of the metal induced toxicity cDNA microarray assays were performed in parallel cultures of the wild type and null MEFs. Preliminary results suggest that a subset of genes is differentially regulated in the wt cells. These results suggest that HIF1α plays an important role in metal induced toxicity.

1472 GENECHIP MICROARRAY ANALYSIS PROVIDES INSIGHT INTO THE DISRUPTIVE EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) ON UROGENITAL SINUS (UGS) AND PROSTATE DEVELOPMENT.

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Mouse prostate development starts as projections (buds) of UGS basal epithelial cells into the surrounding mesenchyme on Gestation Day (GD) 16. *in utero* TCDD exposure blocks ontogenesis of ventral buds, reduces dorsolateral bud number, and delays anterior and dorsolateral bud formation. To investigate the mechanism by which TCDD disrupts UGS development, we examined gene expression in the UGS. Pregnant C57Bl/6J mice were given 5 μg TCDD/kg or vehicle on GD 13. On GD 16, UGSs from male fetuses were separated into ventral and dorsal mesenchyme and epithelium. Total RNA preparations were pooled to make one composite sample per tissue and treatment group. Probes were generated and hybridized onto Affymetrix Murine Genome U74A and U74Av2 arrays. Using Microarray Suite software, 195 genes were identified whose steady state mRNA levels were altered by TCDD in at least one region of the UGS. Forty-three are ESTs without known function. Forty-one genes belong to cytoskeleton and extracellular matrix families. Cell adhesion molecules involved in skin development were also altered, suggesting a major disruption of UGS epithelial development/organization. Twenty-nine altered genes (neural-specific intracellular structure, neural crest markers, and neuropeptides) are associated with neural development. TCDD-AhR target genes were also activated. Five transcription factor genes were altered, of which HNF-3 and capsulin are involved in prostate development. Only 7 altered genes belong to growth factor signaling pathways, of which only TGFβ is known to be important to prostate development. Growth factors may not be important at this early stage of UGS development, or spatial distribution may be more important than amount. Preliminary results from real time LightCycler RT-PCR support the GeneChip results. These findings enhance our understanding of TCDD disruption of fetal UGS development and suggest new research directions, e.g. neural crest cell distribution and function. (NIH Grant ES01332 and Fulbright Grant)

1473 DIFFERENTIATION BETWEEN SENSITIZERS AND IRRITANTS IN THE LOCAL LYMPH NODE ASSAY BY GLOBAL EXPRESSION PROFILING.

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The objective of this study was to use the local lymph node assay (LLNA) in conjunction with high density DNA microarrays to 1) evaluate expression profiling as a technique to discriminate between structurally diverse sensitizers and irritants; and 2) identify potential transcript biomarkers specific for each compound class. Expression profiling was performed on auricular lymph node RNA isolated from female CBA/J mice (n=8/group) dosed with either 4:1 acetone/olive oil (vehicle control) or one of four sensitizers (0.25% dinitrochlorobenzene, DNCB; 0.05% oxazolone, OX; 25% trimellitic anhydride, TMA; 1% toluene diisocyanate, TDI) or one of four irritants (25% chlorobenzene, CB; 5% phenol, PH; 20% benzoic acid, BA; 0.5%, 1%, or 2% benzalkonium chloride, BAC) according to the standard LLNA protocol. All experiments were performed using 12, 000-gene Affymetrix arrays. Two-dimensional agglomerative cluster analysis revealed a statistically significant similarity in the expression profiles of sensitizer-treated animals ($R^2 > 0.6$; $P < 0.01$). Irritant profiles clustered together in an unrelated group with no correlation to the sensitizer cluster ($R^2 < 0$). Evaluation of sensitizer-specific sig-

natures revealed a strong induction of multiple cell cycle and inflammatory response transcripts that are unaltered by irritant treatment. These clusters of potential biomarkers effectively confirm proliferation-based scoring of sensitizers in the LLNA. Expression profiling performed on higher doses of BAC that typically score positive in the LLNA revealed a similar induction of many of these same cell cycle and inflammatory-response transcripts, providing a molecular basis for high-dose irritant scoring in the LLNA. These results illustrate the utility of query-able expression profile databases for compound classification, and revealed several candidate biomarkers that may serve as sensitive endpoints for the LLNA.

1474 DNA ARRAY ANALYSIS OF GENE EXPRESSION ALTERATIONS UNDERLYING SENSITIZATION USING THE LOCAL LYMPH NODE ASSAY AS A MODEL.

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The objective of this study was to identify the underlying and distinctive genetic mechanisms of dermal and respiratory sensitization using the local lymph node assay (LLNA) and DNA array technology. We have previously shown that there are temporally dependent alterations in gene expression in mouse auricular lymph nodes at days 1, 3, and 6 of dosing with a known dermal sensitizer, 1-chloro-2, 4-dinitrobenzene (DNCB). The current study was designed to 1) examine early gene expression changes following exposure to DNCB, as well as a known respiratory sensitizer, trimellitic anhydride (TMA); and 2) identify markers to discriminate between dermal and respiratory sensitization. Female CBA/J mice were dosed with either 4:1 acetone:olive oil (AOO) vehicle-control, 0.25% DNCB, or 25% TMA. Groups of vehicle-control (n=8), DNCB-treated (n=8), and TMA-treated (n=8) mice were sacrificed and auricular lymph nodes (AULS) were removed and pooled within each group 1, 4, 12, and 24 hours following a single dose of either DNCB or TMA. An additional group of naive (n=9) mice were sacrificed and AULS were removed and pooled at the 24-hour time point. Total RNA was isolated and cDNA was prepared and radiolabeled using gene-specific primers to probe a murine cDNA expression array (Atlas™ Mouse 1.2 Array; Clontech Labs, Inc.). Of the arrayed genes, DNCB-induced changes in expression levels were detected in approximately 0.8%, 0.3%, 3.4%, and 0.2%, and TMA-induced changes were detected in 1.2%, 1.1%, 3.1%, and 1.8% at 1, 4, 12, and 24 hours, respectively. Unrelated gene expression signatures between DNCB and TMA were revealed. These data demonstrate the differential and temporally dependent gene regulation that occurs following the topical application of a known dermal or respiratory sensitizer. Additionally, these data illustrate the utility of expression profile databases to distinguish dermal from respiratory sensitizers that are traditionally indistinguishable in the LLNA.

1475 cDNA MICROARRAY ANALYSIS OF GENE EXPRESSION DURING CHEMICAL INDUCED NEPHROCARCINOGENICITY IN THE EKER RAT.

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The Eker rat, derived from the Long-Evans strain, provides an excellent model for studying renal carcinogenesis. A germ-line insertion in the tuberous sclerosis (*Tsc-2*) tumor suppressor gene, predisposes these rats to the development of spontaneous or chemical induced tumors. Hydroquinone (HQ) is a rodent carcinogen and a possible human carcinogen. 2, 3, 5-*tris*-(glutathion-*S*-yl)hydroquinone (TGHQ) has been implicated as a reactive metabolite that contributes to HQ-mediated nephrocarcinogenicity. The mechanisms of HQ-mediated renal carcinogenesis remain unknown. TGHQ-treated rats develop numerous toxic proximal tubular dysplasias that represent early transformation within tubules, and adenomas are seen to arise from these lesions. cDNA microarrays were used to examine aberrant gene expression associated with TGHQ-induced transformation in tumor tissue derived from the kidneys of Eker rats treated with TGHQ. The expression of 38 genes were altered by three-fold or greater in tumor tissue compared to normal kidney tissue. Furthermore, the levels of a number of genes, identified using cDNA microarrays, were validated by semi-quantitative RT-PCR. The differentially expressed genes are involved in cell cycle regulation (cyclin-dependent kinase 4), signal transduction (extracellular regulated kinase 2), stress response (glutathione-S-transferases), tissue remodeling (urokinase-type plasminogen activator) and energy production (cytochrome c oxidase), and likely contribute to tumor development in the Eker rat. Importantly, the annexin I gene, implicated in signal transduction, was highly induced at both the mRNA and protein levels in tumor tissue. In addition, toxic tubular dysplasias obtained by laser capture microdissection of kidneys from Eker rats treated chronically (10 months) with TGHQ also show high mRNA levels of annexin I (6-fold over control). These results suggest that annexin I gene likely plays an important role during the early stages of TGHQ-mediated nephrocarcinogenicity in the Eker rat. (GM 39338, ES 07784).

1476 NBS1 CANCER-SUSCEPTIBILITY GENE INFLUENCES GENE EXPRESSION FOLLOWING DNA DAMAGE.

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The contribution of defects in cellular response to DNA damage and development of disease, particularly cancer, in patients with genetic susceptibilities is poorly understood. Nijmegen Breakage Syndrome (NBS), a chromosome instability disorder, is associated with hypersensitivity to ionizing radiation and predisposition to cancer. Nibrin, the gene product of NBS1, complexes with Rad50/MRE11 and co-localizes with BRCA1 to regions of damaged DNA, suggesting a role in normal DNA damage recognition/repair processes. We hypothesize that nibrin is important in pathways regulating cell cycle checkpoints in response to DNA damage downstream of, but complementary to, ATM-dependent signaling. We investigated cell cycle checkpoint responses and gene expression changes in mortal diploid fibroblasts in response to ionizing radiation (1.5 to 5 Gy), comparing nibrin-deficient (NBS-/-) and wild type (WT) cells. Three assays were used: (i) flow cytometric analysis of BrdU incorporation in DNA synthesis to investigate G1/S checkpoint response, (ii) fluorescence-based DAPI staining to quantitate mitotic cells, a measure of G2/M checkpoint response, and (iii) gene expression profiling, to elucidate molecular pathways involved in checkpoint responses that are defective in NBS. We observe a mitotic delay defect in NBS-/- cells following exposure to low doses of gamma irradiation, indicating a role for NBS1 in the G2/M checkpoint. In contrast, at higher doses (5 Gy), NBS-/- cells induce a G2/M checkpoint delay. This suggests an important role for nibrin in checkpoint responses and repair at low levels of DNA damage, but at higher levels the activation of compensatory pathways leads to a stronger checkpoint delay. Preliminary comparison of responses to radiation induced damage in WT and NBS-/- cells, using cDNA microarray analysis, revealed nibrin-dependent transcripitional patterns that may be correlated with defects in cell cycle checkpoints and DNA repair.

1477 APPLICATION OF TOXICOGENOMICS TO DRUG DISCOVERY AND DEVELOPMENT: EFFECT OF A KINASE INHIBITOR ON GENE EXPRESSION IN RAT NASAL TISSUE.

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Kinase inhibitors are important therapeutic targets. Because of the large number of intracellular kinases, specificity of a selected kinase inhibitor is crucial for its pharmacological action as well as its potential toxicity. Within a class of kinase inhibitors, toxicity may be similar, but not likely identical. Compound X is a kinase inhibitor that was being developed for treatment of asthma. In a 5-Day oral toxicity study in rats, compound X was shown to produce nasal epithelial toxicity. The aim of this work is two fold: 1- Examine the use of toxicogenomics to evaluate alteration in gene expressions in the nasal tissue as a function of treatment with an attempt to elucidate the mechanism(s) of toxicity. 2- Determine the overall utility of toxicogenomics in drug discovery and development. Male rats were dosed with Compound X at 0, 100 and 1000 mg/kg/day for 1 or 5 days. Histopathologic evaluation confirmed target organs. Toxicogenomic analyses utilized the rat RgU34A chip(Affymetrix). Treatment-related nasal epithelial degeneration and inflammation in the transitional, respiratory and olfactory mucosa was detected in some rats in the high dose group after 1 or 5 days of treatment. Principal component analysis of individual rats showed that the affected rats were clustered separately. Specific genes involved in inflammation, cell proliferation, apoptosis and cell injury were up regulated while genes involved in neuronal transport and signaling, and drug metabolizing enzymes were down-regulated. The alteration in gene expression patterns correlated with treatment-related histopathologic changes.

1478 CHARACTERIZATION OF THE MOLECULAR MECHANISMS UNDERLYING THE HEPATOCARCINOGENESIS INDUCED BY THE NONGENOTOXIC CARCINOGENS, PEROXISOME PROLIFERATORS, IN THE RAT.

C. Michel, J. P. Marchandeau, M. Flor, D. Leroy, J. C. Gautier and E. Boitier. *Aventis Pharma, Drug Safety Evaluation, Vitry sur Seine, France.* Sponsor: M. Bonnefoi.

Peroxisome proliferators (PP), a diverse group of nongenotoxic compounds, cause cell proliferation, apoptosis suppression and peroxisome proliferation through the activation of the peroxisome proliferator-activated receptor alpha (PPAR-alpha). This cascade of events is believed to contribute to formation of liver tumors in rodents. The aim of this study is to evaluate and characterize the molecular mechanisms of the carcinogenesis induced by these compounds and to determine whether

a specific gene expression pattern could be elucidated in rat liver for the compound class of PP. Compounds included in this study were the hypolipidemic compounds Clofibrate (25 and 250 mg/kg), Fenofibrate (10 and 100 mg/kg), and WY-14643 (25 and 250 mg/kg). They were administered twice a day to Sprague-Dawley rats for up to 1 and 5 days. In addition to PP, Phenobarbital (PB, 12 and 120 mg/kg) was also tested in this study as a nongenotoxic hepatocarcinogen acting through a mechanism different from PPAR-alpha activation. Hepatic gene expression profiles were generated using Affymetrix microarrays. Gene expression fold-changes after PP or PB treatment were estimated using a powerful in-house-developed software, GECKO2. The transcriptional mapping of the liver was compared with classical toxic end-points such as histopathological evaluation. Preliminary histopathological results indicated a moderate increase in mitotic figures (evocative of an increase in cell proliferation) and mild centrolobular hypertrophy (most likely due to peroxisome proliferation) only with the high dose of WY-14643 after a 5 day-treatment. PB induced a mild hypertrophy (proliferation of endoplasmic reticulum) at the highest dose after 5 days. Initial gene expression analysis clearly revealed distinct molecular patterns between PP and PB. These data should provide mechanistic information on early marker genes whose modulation of expression would indicate the carcinogenic potential of PP.

1479 GP3: GENEPIX POST-PROCESSING SCRIPT FOR AUTOMATED ANALYSIS OF RAW TOXICOGENOMIC MICROARRAY DATA.

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Toxicogenomic studies generate vast amounts of data that require processing prior to clustering and other analyses. We describe a script to automate the post-processing of raw microarray data captured with GenePix, a widely used commercial microarray image analysis application. The script, written in Perl, filters outlying signal intensities, performs background corrections and normalizes signal intensities between the Cy3 and Cy5 channels. Signal intensities are filtered to ensure they exceed a defined signal threshold of detection while also falling below the saturation level of the microarray scanner. After filtering, valid signals undergo local background correction prior to subsequent normalization. Systematic and experimental biases between the two fluor-labeled cDNA populations being compared in a two-color fluorescence-based cDNA microarray assay can result in inaccurate quantitation of relative differences in gene expression. To minimize this affect, signal intensities for each fluor are adjusted to normalize the gene expression distribution in log2 space. By doing so, signal intensities across the entire microarray can be expressed as a percentage thereby minimizing systematic biases in fluor incorporation and intensity characteristics and differences in RNA quality and sample handling. The script can operate in batch mode to process data in a high-throughput manner. Other features include abilities to override default parameters of the script through a command line interface. Filtered and normalized values are appended to the GenePix results file. In addition, averages and standard deviations of replicate spots are calculated and provided in a summary file. Therefore this script automates the high-throughput post-processing of raw toxicogenomic microarray data generated by GenePix and minimizes human error introduced with repetitive manual adjustments.

1480 DEVELOPMENT OF A RELATIONAL TOXICOGENOMICS DATABASE FOR THE PREDICTION OF CHEMICAL TOXICITY.

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Toxicogenomic studies generate vast amounts of data that require proper storage in order to facilitate data handling and analysis. The inability to relate changes in global gene expression to specific genes, their function, and other information further complicates data analysis and interpretation. To automate these essential functions, dbZach, a database operating under the Oracle relational database management system has been built. dbZach provides a core database that handles all aspects of our multi-species toxicogenomic/microarray investigations including: 1) storage of important cDNA/EST clone information, such as sequence, 2) frequent, automated updates of gene annotation information, 3) microarray data storage, 4) physical mapping data for each microarray utilized and constructed within the Lab.. Currently, dbZach is comprised of four main subsystems: 1) the Clones Subsystem, 2) the Microarray Subsystem, 3) the Gene Function Subsystem, and 4) the Lookup Subsystem. The subsystems interrelate to find data within a dataset, and define relationships between tables. Querying dbZach for information regarding mTZ2.0, our mouse microarray, reveals the availability of 2, 288 cDNAs/ESTs representing 1, 948 unique genes. Ninety-five percent of the available cDNAs/ESTs can be assigned to a specific cluster with the average cluster represented by 1.25 clones. Less than 1% of the genes on our murine microarray, mTZ2.0, are represented by more than 3 clones. As part of our effort to develop a transcript profile database predictive of chemical toxicity, future planned developments for dbZach include compliance with MIAME standards for microarray experiments, statistical analysis functionality, and comparisons of global gene expression data stored within dbZach.

1481 UTILIZATION OF SUBTRACTIVE HYBRIDIZATION AND MACROARRAYS TO DETERMINE ALTERED GENE EXPRESSION IN PYRENE EXPOSED MUMMICHOG *(FUNDULUS HETEROCLITUS).*

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Mummichogs are ideal for monitoring the sublethal effects of pollutants in estuarine environments along the east coast because of their small home range. Estuaries, due to their proximity to humans are susceptible to anthropogenic stress from numerous sources, such as polycylic aromatic hydrocarbons (PAHs), including pyrene. Therefore, mummichogs were exposed in the laboratory to several concentrations of pyrene at 0, 10, 20, 35, and 50 ppb in 7-day static renewal exposures and alterations in gene expression in liver was examined. Previous work in our laboratory has demonstrated that pyrene induces XIR retrotransposon and P450 1A RNA levels. Subtractive hybridization was performed on mRNA from the pyrene exposed mummichog to enhance the production of differentially expressed transcripts and to find novel biomarkers of PAH exposure in mummichog. The transcripts were cloned and 96-gene macroarrays were produced. Control and treated mummichog liver cDNA was then incubated onto the blots and changes in gene expression were measured. Genes demonstrating differential expression due to pyrene will be sequenced and presented at SOT.

1482 DEVELOPMENT OF MICROARRAYS AS A TOOL FOR DISCOVERING ENVIRONMENTAL EXPOSURE INDICATORS.

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Toxicogenomics is research that can be applied to identification of differential gene expression in wildlife and predict effects of environmental stressors. We are developing a multiple stressor exposure model using fathead minnow (Pimephales promelas) cDNAs in a microarray platform. A two-pronged approach has been developed to examine specific gene expression and to identify patterns over a portion of the expressed genome through the use of subtractive libraries. In order to start synthesis of a fathead minnow specific gene chip for array analysis, homologs of specific genes were identified in P. promelas using degenerate PCR primers based on conserved homology across mammalian and non-mammalian taxa. Categories of genes include DNA repair genes, receptor and non-receptor kinase genes, transcription factors, and genes involved in apoptotic cascades such as caspases. Initially, single exposure-specific microarrays were employed using subtractive cDNA clones to identify gene patterns for single-exposure analysis. Multiple exposures can also be assessed at the microarray level to gain a better understanding of relative bioavailability of environmental stressors present in mixtures. Thus, the use of microarray technology to examine gene expression is a powerful tool in understanding the impacts of environmental exposures.

1483 NCBI-BASED ANNOTATION AND CROSS–COMPARISON OF MICROARRAY CHIP FEATURES.

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Transcript profiling (TxP) uses genomic technologies such as microarrays to measure the response of hundreds to thousands of transcripts to various environmental perturbations. These studies are carried out to determine not only the response of certain genes, but also overall patterns of gene expression. A toxicological mechanism may be inferred from the response of a particular transcript, or from the pro-

file of the responses for hundreds of genes. However, comparison of data from different laboratories is complicated not only by the nature of the different microarray technologies used, but also by the inconsistent annotation used by different platforms to describe the sequences queried. As a tool for the multi-laboratory genomics research program coordinated by the ILSI Health and Environmental Sciences Institute, we developed a system using custom Perl programs and data from the NCBI (National Center for Biotechnology Information). The accession number for a sequence queried by a microarray element is used to obtain cross-referenced information from UniGene (a system for grouping GenBank sequences into gene-oriented clusters) and/or LocusLink (curated descriptive information about genes). The UniGene and LocusLink ID s provide a means to determine if sequence elements on the same or different platforms monitor the same gene, and provide a source of consistent annotation. Thus, of the 20, 151 "genes" on the Affymetrix U34 Rat Genome GeneChip®, 1806 are shared in common with the 5902 "genes" on the Incyte RatGem 3 microarray. Likewise, the U34 GeneChip has 232 "genes' in common with Phase-1 Toxicology's 250 "gene" microarray. While cross-referencing elements based on accession number alone is not precise, this system does provide a rapid method for determining some similarities and a means for updating annotation on a regular basis.

1485 STUDIES ON THE MECHANISM OF ACTIVATION OF 7H-BENZO[c]FLUORENE.

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BENZO(C)FLUORENE (B[c]F) induces lung tumors in A/J mice when administered by ip injection or in the diet. B[c]F is responsible for a major DNA adduct in lung of mice exposed to a variety of coal tar materials. This study evaluates the DNA adducting properties of B[c]F metabolites in order to better understand the mechanism by which B[c]F induces lung tumors. B[c]F, 7-hydroxy B[c]F, 3-hydroxy B[c]F, or 3, 4-dihydrodiol B[c]F were synthesized and evaluated for their ability to form DNA adducts in skin and lung of mice following topical administration. B[c]F or a metabolite was applied to the shaved backs of female CD1 mice at 1.0 and 4.0 umol dose levels. Skin and lung tissue were collected 24 hr after dosing and DNA was evaluated using 32P-postlabeling analysis. DNA adduct formation was detected in skin and lung of mice treated with B[c]F. These DNA adduct maps were similar to those observed in lung when B[c]F is administered to mice in the diet. The 3, 4-dihydrodiol metabolite of B[c]F formed adducts that were similar to the pattern observed with B[c]F. The total level of lung DNA adducts observed with the 3, 4-dihydrodiol metabolite was considerably greater then that observed with B[c]F. These results suggest that B[c]F metabolic activation likely involves the formation of the 3, 4-dihydrodiol metabolite, which could be further converted to a dihydrodiol epoxide metabolite. These results are consistent with the known activation pathway of other more extensively study carcinogenic polycyclic aromatic hydrocarbons. (This research was supported by funds from EPRI.)

1486 ^{32}P-POSTLABELING OF PHOTOCHEMICALLY BOUND DNA ADDUCTS.

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Many compounds can be photoactivated to genotoxic products. We have investigated three photoactive compounds by ^{32}P-postlabeling for DNA adducts: BAY y3118, an experimental fluoroquinolone antibiotic, chlorpromazine (CPZ) an antiemetic and antipsychotic and 4, 5', 8-trimethylpsoralen (TMP) a compound related to 8-methoxypsoralen used in the PUVA treatment of psoriasis. BAY y3118 has not been investigated for DNA adduct formation, although it is photochemically mutagenic (*Arch. Toxicol.*, **74**, 555, 2000), while CPZ is known to form DNA adducts *in vitro* (*Chem. Biol. Interact.*, **51**, 273, 1984) and TMP has been previously demonstrated by ^{32}P-postlabeling to form adducts in HeLa cells (*Carcinogenesis*, **15**, 89, 1994) in the presence of UV light. All three compounds were photoactivated by 345 nm UVA light in the presence of calf thymus DNA and the DNA purified by ethanol precipitation. Photoactivation of BAY y3118 yielded DNA that exhibited substantial fluorescence (Ex 350 nm, Em 450 nm). Such modification did not occur when BAY y3118 was irradiated prior to mixing with the DNA suggesting the formation of a short lived species during the photochemical reaction. Using ^{14}C-BAY y3118, photoactivation resulted in radioactivity bound to DNA corresponding to about 70 adducts in 10^6 normal nucleotides. Oxygen was not required since, under anaerobic conditions, the fluorescence associated with the purified DNA was even higher. Postlabeling of these DNA samples either directly, using nuclease P_1 or column (Water Oasis HLB) enrichment of the samples showed DNA adducts were formed with CPZ but not with the other two chemicals. TMP, however, was positive by the specific approach developed for it which involves digestion to di-2'-deoxyribonucleoside monophosphates (*Carcinogenesis*, **15**, 89, 1994), but even using these conditions, BAY y3118 was negative. Thus, although successful with CPZ and TMP, we have not detected DNA adducts by ^{32}P-postlabeling with BAY y3118. (Supported by NCI Grant RO1-CA86056)

1487 APURINIC/APYRIMIDINIC (AP) SITES, INTERMEDIATES IN BASE EXCISION REPAIR, ARE NOT INCREASED BY ETHYLENE OXIDE INHALATION IN RATS.

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Ethylene oxide (EO) is an important industrial chemical and gaseous sterilant that is mutagenic and carcinogenic in rodents. N7-(2-hydroxyethyl) guanine (HEG) represents 90% of the DNA adducts induced by EO. While not promutagenic itself, HEG could cause mutations through the formation of apurinic (AP) sites, which are promutagenic lesions. We developed a sensitive assay to detect AP sites in genomic DNA, which can quantitate <1 AP site/10^6 nucleotides. A newly established AP site cleavage assay can further characterize three kinds of AP sites (3'-nicked, 5'-nicked and intact). The present study was conducted to quantitate the number of AP sites in brain, liver, lung and spleen in male rats exposed by inhalation to 0 and 100 ppm EO (a concentration with positive results in HPRT mutation and 2-year carcinogenicity studies) for up to 4 weeks. Furthermore, the AP sites in spleen, a target organ for mutation and carcinogenicity studies, were characterized using the cleavage assay in male rats exposed to a single 6 hour or 4 weeks of exposure. The number of AP sites in each organ of control and EO exposed rats were shown in Table 1. No significant differences in the number of AP sites were observed between 0 and 100 ppm EO. AP site analysis of spleen using the cleavage assay showed that the majority of AP sites were 3'-nicked AP sites in both control and EO exposed rats. No significant differences were observed between control and exposed rats. These data suggest that AP sites induced following EO are efficiently repaired and that AP sites are not likely to be the primary mechanism for mutations and carcinogenesis induced by EO. Table 1 Number of AP sites (per 10^6 nucleotides, n=8, mean ± S.D.) in DNA of rats exposed by inhalation to 0 and 100ppm EO.

Groups	Brain	Liver	Lung	Spleen
Control (0 ppm)	6.3±1.85	6.1±0.71	9.6±1.41	7.7±1.95
EO exposed (100 ppm)	7.6±3.39	6.5±1.08	7.5±1.42	8.6±1.44

1488 ESTROGENIC PHENOL AND CATECHOL METABOLITES OF PCBs DOWN REGULATE CATECHOL-O-METHYLTRANSFERASE.

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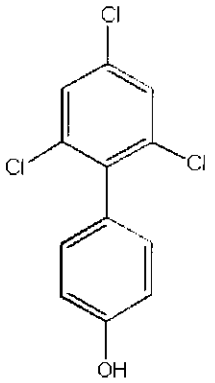
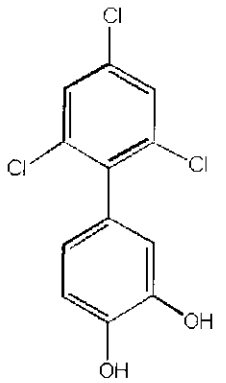
PCB mixtures induce liver tumors in female rats and this effect may be due altered estrogen metabolism. This laboratory has demonstrated that metabolites of PCBs are potent inhibitors of COMT and may contribute to reduced clearance of genotoxic catechol estrogens (CEs). Liver S9 of female SD rats treated with carcinogenic doses of Aroclor 1254 for 21 days (10 mg/kg/day) showed ca. 40% reduction of COMT activity towards CEs. We hypothesize that estrogenic PCB metabolites may contribute to reduced COMT activities *via* the estrogen receptor, which has been demonstrated to regulate COMT. The xenoestrogens, 2, 4, 6-trichloro-4-biphenylol and 2, 4, 6-trichloro-3', 4'-biphenyldiol were incubated in human MCF-7 cells expressing the ER and COMT for 48 hours and expression of COMT determined. Western blot analysis showed that COMT protein levels were reduced in a concentration dependent manner by both metabolites. Additionally RT-PCR analysis showed a concentration dependent reduction of COMT RNA, suggesting that the PCB metabolites reduced COMT expression at the transcriptional level. The above suggests that COMT levels may be reduced *via* interaction between PCB metabolites and the ER. The above information, coupled with our prior work, supports the hypothesis that PCB metabolites may contribute to PCB-mediated

carcinogenesis through reduced clearance of endogenous and xenobiotic catechols by reducing both catechol O-methyl transferase levels and activities.



2,4,6-trichloro-4-biphenylol



2,4,6-trichloro-3',4'-biphenyldiol

1489 FUNCTIONAL CHARACTERIZATION OF N-ACETYLTRANSFERASE 1 (NAT1) AND 2 (NAT2) IN MAMMARY PRIMARY CELL CULTURES FROM RAPID AND SLOW ACETYLATOR SYRIAN HAMSTER CONGENIC AT NAT2.

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Aromatic and heterocyclic amine carcinogens present in the diet and in cigarette smoke induce breast tumors in rodents. N-acetyltransferase-1 (NAT1) and N-acetyltransferase-2 (NAT2) are important in the metabolism of these carcinogens. Human epidemiological studies suggest that genetic polymorphisms in NAT1 and/or NAT2 predispose to breast cancer in women exposed to these carcinogens. We hypothesized that NAT1- and NAT2-catalyzed activation and/or deactivation of these carcinogens within the breast may effect breast cancer risk. Our laboratory constructed a rapid and slow acetylator Syrian hamster model congenic at the NAT2 locus that we used to investigate NAT1 and NAT2 expression in mammary primary cell cultures. p-Aminobenzoic acid (PABA), and 4-aminobiphenyl N-acetyltransferase and N-hydroxy-2-amino-1-methyl-6-phenylimidazo[4, 5-b] pyridine O-acetyltransferase activities were significantly ($p<0.05$) higher in mammary cell cultures from rapid than from slow acetylator hamsters. Sulfamethazine (an NAT1-selective substrate) N-acetyltransferase activity was low and did not differ between rapid and slow acetylators. NAT1 and NAT2-specific mRNA were quantitated by real-time polymerase chain reaction. Levels of NAT1 and NAT2 mRNA were similar, but NAT2 mRNA was higher in rapid than in slow acetylators. Hamster mammary cells cultured in the medium supplemented with PABA for 24h showed a significant decrease (50-70%) in NAT2 activity. The loss of activity was concentration-dependent (EC50 4 *micro*M) and selective because PABA treatment showed no effect on NAT1 activity. The loss of NAT2 activity was associated with reduction in the amount of NAT2 but not NAT1 mRNA. These studies clearly show NAT1- and NAT2 -catalyzed acetylation of aromatic and heterocyclic amine carcinogens in mammary cell cultures modified by substrate regulation of NAT2 expression. Partially supported by USPHS grant CA-34627 from the National Cancer Institute.

1490 N-ACETYLTRANSFERASE 2 PHENOTYPE IN PAINTERS WITH UROTHELIAL BLADDER CANCER AND NON-DISEASED COLLEAGUES.

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Aim: This study was designed to evaluate the impact of N-acetyltransferase 2 (NAT2, substrate: aromatic amines) on bladder cancer in painters. Background: Until the beginning of the 60s, painters in Germany have used, among others, azo dyes based on carcinogenic aromatic amines. Materials and Methods: Sixteen painters, from different areas in Germany suffering from transitional cell carcinoma of the bladder, and 26 healthy painters from the same areas and at the same age (± 5 years) as controls, were investigated. All painters were phenotyped for NAT2 by the molar ratio of two caffeine metabolites in urine, determined by a HPLC method. All painters were asked for the period of work as a painter and for their life-time smoking habits. Results: Exposure against paints (colorants) started in 1950 in cases (SD 9.3) and controls (SD 9.6). Fourteen cases and 23 controls had been exposed to paints before 1960. Age at first exposure against paints was 15.5 years (SD 5.3) in cases and 16.3 years (SD 4.9)in controls. Cases had worked 31.1 years (SD 15.0), and controls had worked 44.8 years (SD 7.2) as a painter. Four cases and 7 controls were non-smokers. In the present study, 88% of 16 diseased painters and 65% of 26 non-diseased painters were of the "slow" acetylation phenotype. Conclusions: The results suggest that the "slow" acetylation status is an individual risk factor for bladder cancer in persons occupationally exposed to amounts of carcinogenic aromatic amines released from water-soluble azo dyes.

1490A N-ACETYLTRANSFERASE-1 AND -2 GENOTYPES AND PROSTATE CANCER.

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N-acetyltransferase-1 (NAT1) and N-acetyltransferase-2 (NAT2) are important in the bioactivation and deactivation of aromatic and heterocyclic amine carcinogens that induce prostate tumors in the rat. We investigated the association of genetic polymorphisms in *NAT1* and *NAT2*, alone and in combination, with human prostate cancer. Incident prostate cancer cases and controls in a hospital-based case-control study were frequency-matched for age, race, and referral pattern. Slow acetylator *NAT2* genotype exhibited borderline association with prostate cancer (OR, 1.75; 95% CI, 0.86-3.54), but the association was greater for individuals homozygous for *NAT2* alleles (*NAT2*5*) with lowest NAT2 catalytic activity (OR, 2.43; 95%CI, 0.96-6.12; P = 0.08). In contrast, individuals possessing the putative rapid acetylator *NAT1* genotype (one or more *NAT1*10* allele) were over-represented in prostate cancer (OR, 2.17; 95% CI, 1.08-4.33; P= 0.03). Combinations of *NAT1*10* with *NAT2* slow genotypes (OR, 5.08; 95% CI, 1.56-16.5; P= 0.008) or with *NAT2* very slow acetylator (homozgyous *NAT2*5*) genotype (OR, 7.50; 95% CI, 1.55-15.4; P = 0.016) further increased prostate cancer risk. The results of this pilot study suggest increased susceptibility to prostate cancer in combinations of *NAT1*10* and the slowest *NAT2* genotypes, but needs to be investigated further in larger cohorts and in other ethnic populations. Partially supported by USPHS grant CA34627 from the National Cancer Institute.

1491 THE PRESENCE OF DIMETHYLARSINOUS ACID (DMA^{III}) IN THE URINE OF RATS TREATED WITH DIMETHYLARSINIC ACID (DMA).

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DMA is carcinogenic to the rat urinary bladder when administered in the diet or drinking water. At a dietary dose of 100 ppm, it produces cytotoxicity within six hours and increased proliferation (hyperplasia) by seven days of administration. 2, 3-Dimercaptopropane-1-sulfonic acid (DMPS) reacts with trivalent forms of arsenic and has been used in the treatment of arsenic poisoning. When DMPS is co-administered with DMA the cytotoxicity and regenerative hyperplasia of the urothelium are inhibited. Analyses of arsenicals in 24-hr urine specimens showed high concentrations of DMA and trimethylarsine oxide (TMAO) in the DMA-treated group. DMA levels increased and TMAO levels decreased in the group co-administered DMA and DMPS. The presence of TMAO suggests the formation of the intermediate trivalent metabolite, DMA^{III} since DMA must be in the trivalent state prior to methylation to TMAO. We hypothesize that formation of the reactive intermediate, DMA^{III}, is involved in the induction of the urothelial cytotoxicity found in DMA-treated rats. To evaluate this possibility, groups of 10 female 5-week old Fischer F344 rats were fed either DMA, DMA plus DMPS, or the control diet. Fresh void urines were collected from the rats approximately 24 hr after treatment began and again during week 10 of the study. DMA^{III} was found in the urine of DMA and DMA plus DMPS-treated rats at approximately micromolar concentrations (24 hr:DMA-1.4±0.3; DMA+DMPS-0.9±0.2; wk 10:DMA-5.0±1.2; DMA+DMPS-3.0±0.2). Using rat (MYP3) and human (1T1) cells, we further evaluated the cytotoxicity of various forms of arsenic. Sodium arsenite, monomethylarsonous acid (MMA^{III}) and DMA^{III} were cytotoxic at 0.4-5 μM concentrations, whereas MMA, DMA, and TMAO were cytotoxic at millimolar concentrations. Since the urinary DMA^{III} from the rats fed 100 ppm DMA is present at micromolar concentrations, these results suggest that DMA^{III} produced *in vivo* may be responsible for the toxic effects in the rat urinary bladder after dietary administration of DMA.

1492 PREDICTION OF THE CARCINOGENESIS OF FUMONISIN B_1 IN MALE AND FEMALE F344/N/NCTR RATS FROM URINARY AND TISSUE SPHINGOLIPID CHANGES.

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Fumonisin B_1 (FB1) is a mycotoxin that has been detected world-wide as a result of growth of *Fusarium* species in corn. FB1 was carcinogenic in a two-year feeding study inducing renal tubule tumors in male F344/N/NCTR rats at 50 and 150 ppm, but was not carcinogenic at the highest dose (100 ppm) in females. FB1 inhibits *de novo* sphingolipid synthesis through inhibition of ceramide synthase, resulting in increased tissue levels of sphinganine (Sa). This increase has been used as a biomarker of exposure. Urinary sphingoid bases were determined in the feeding study after 6, 10, 14, and 26 weeks, and tissue levels of sphingoid bases were determined at the interim sacrifices and in some cases at 2-years. Urinary Sa was increased in the males at 15, 50, and 150 ppm, and in the females at 50 and 100 ppm. Urine Sa/sphingosine (Sa/So) ratios were likewise consistently increased in the males at 15, 50, and 150 ppm, and in the females at 50 and 100 ppm. Kidney Sa and Sa/So were elevated in the males at 15, 50, and 150 ppm, and in the females consistently only at 50 and 100 ppm. Liver Sa/So ratios were only elevated at the interim sacrifices in the female rats at 100 ppm. These results demonstrate that induction of tumors in the kidney but not in the liver of male rats correlates with Sa and Sa/So ratios in these tissues. These results also show a correlation between the renal tubule carcinogenicity of FB1 and the extent of accumulation of Sa in the male rat urine and kidney; however, since Sa levels were also increased in the female urine and kidneys in the absence of carcinogenicity, the Sa biomarker may not be predictive of tumorigenic outcome in the kidney.

1493 THE EFFECT OF ETHANOL IN THE PROLIFERATION OF BOTH ESTROGEN SENSITIVE LEYDIG CELLS AND ESTROGEN RECEPTOR POSITIVE MCF-7 CELLS.

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Excess alcohol consumption has been identified as a risk factor for breast and other hormonal cancers. Now alcohol is considered as a co-carcinogen. The mechanism for increased of hormonal cancers is not known. Recently it has been reported that ethanol induces proliferation of MCF-7 cells and increases estrogen receptor expression. While the mechanism for these effects are not known, they seem to support epidemiological evidence that link alcohol consumption with an increased risk of breast cancer. In this study we have examined the influence of low and high concentrations of ethanol on testicular Leydig cells and MCF-7 cells. We examined the effect of ten different concentrations of ethanol on the proliferation of TM3 Leydig and MCF7 cells. We report for the first time that exposure of cells to a low dose of ethanol (1 ml/ml) produce significant increase in cell growth, and the doses of ethanol greater then 5 ml/ml produced inhibition of cell growth. Exposure of Leydig cells to ethanol in combination with tamoxifen failed to significantly lower the ethanol-mediated proliferation. It appears that alcohol may be playing a role in hormonal carcinogenesis presumably through influencing signaling pathways involved in the regulation of cell cycle.

1494 INHIBITION OF ESTROGEN RECEPTOR NEGATIVE BREAST CANCER CELL GROWTH BY SELECTIVE ARYL HYDROCARBON RECEPTOR MODULATORS.

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Aryl hydrocarbon receptor (AhR) agonists induce inhibitory AhR-estrogen receptor α (ERα) crosstalk in breast cancer which is accompanied by inhibition of cell cycle progression *in vitro* and tumor growth in rodent models. Growth of ER-negative MDA-MB-468 breast cancer cells is also inhibited by AhR agonists and in Balb/c athymic nude mice bearing MDA-MB-468 cell xenografts, the selective AhR modulators (SAhRMs) 6-methyl-1, 3, 8-trichlorodibenzofuran (MCDF) and diindolylmethane (DIM) inhibited mammary tumor growth. We have further investigated the Ah-responsiveness of several other ER-negative breast cancer cell lines and the growth inhibitory effects of MCDF, DIM and related SAhRMs. Cell lines were initially screened for induction of CYP1A1-dependent ethoxyresorufin *O*-deethylase (EROD) activity after treatment with 10 nM TCDD for 24 hr. The results showed that EROD activity was significant induced by TCDD in HCC-38, MDA-MB-453, MDA-MB-157, and MDA-MB-435 cells, whereas no induction was observed in BT-20 cells. In parallel studies, the effects of TCDD, MCDF and DIM on proliferation of the four ER-negative breast cancer cell lines were also investigated. TCDD and the SAhRMs inhibited growth of the four ER-negative breast cancer cell lines. For example, the IC_{50} values for inhibition of MDA-MB-157 cell growth using 2.5% serum were ≤ 1 nM TCDD, 100-1000 nM DIM and 10-100 nM MCDF. The IC_{50} values for these compounds varied among the cell lines and was also dependent on percent serum used. Current studies are focused on the AhR-mediated mechanisms of cell growth inhibition. (Supported by NIH CA64081, ES04176 and ES09106)

1495 EFFECT OF CHRONIC HEXACHLOROBENZENE (HCB) EXPOSURE ON TELOMERASE ACTIVITY IN HUMAN MAMMARY EPITHELIAL CELLS.

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HCB is a widespread environmental contaminant virtually present in 100% of North American women. Epidemiological studies suggested that a higher HCB level could be a risk factor for breast cancer. HCB has been shown to increase the phosphorylation of the EGF receptor family, such as *neu*, in human mammary epithelial cells. Such epigenetic alterations can promote the appearance of cells with a procarcinogenic phenotype. Normally absent in somatic cells, telomerase is induced in most tumour cells enabling them to divide indefinitely and become immortal. In order to evaluate the potential of HCB to cause epigenetic changes akin to a procarcinogenic phenotype, cultures of a non-tumorigenic human mammary epithelial cell line, MCF10-A, were chronically exposed to 20 µM HCB (0.1% in DMSO) or to DMSO alone. Cells were grown in DMEM supplemented medium with 20 ng/ml EGF, 10 mg/ml insulin, 5% horse serum, and telomerase activity was measured using the telomeric repeat amplification protocol at specific passages. Cell proliferation was assessed throughout the experiment by calculating the population doubling time. Following approximately 25 passages of continuous exposure to HCB, a marked increase in telomerase activity was observed; these cells were proliferating at a 2-fold higher rate. When these cultures were subsequently maintained with DMSO alone, telomerase activity remained higher than in the continuously DMSO-treated cells; the increased cell proliferation also remained higher. The latter observations imply that HCB-induced epigenetic changes are long lasting. Increased telomerase activity is a very widespread phenotype of tumorigenic cells. Overall, results show that a major mechanism enabling mammary epithelial cells to become immortal is activated by continuous HCB exposure. This work supports the contention that environmental contaminants can be a risk factor in breast cancer development.(Supported by the Toxic Substances Research Initiative and the Pgrm of Research in Environment Etiology of Cancer).

1496 EFFECTS OF ORGANOCHLORINES ON c-erbB-2 (*neu*) SIGNALING PATHWAY IN HUMAN MAMMARY EPITHELIAL CELLS.

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Environmental factors such as chemical exposure to contaminants, dietary habits and lifestyle could have an important role in breast cancer development (BCD). Organochlorines are lipophilic contaminants distributed in breast tissue, however their role in BCD is debated. *Neu* is an oncoprotein belonging to the epidermal growth factor receptor (EGFR) family which correlates with poor prognosis for breast cancer patients. In human mammary epithelial cells organochlorines could perturb receptor signaling pathways, such as that linked to the *neu* receptor overexpressed in several breast cancers. This research aims to assess the *in vitro* effect of an environmental mixture (Mix), made of 3 PCBs, 7 polychlorinated dibenzofurans (PCDFs) and 6 polychlorinated dibenzodioxins (PCDDs) found in human breast milk, on constitutive and phosphorylated *neu* levels in a non tumorigenic human mammary epithelial cell line (MCF-10A). Cells, grown in DMEM medium supplemented with 20 ng/ml EGF, 10 µg/ml insulin, 5% horse serum, were exposed to the Mix for 10 and 30 min, then were collected with lyzing buffer for Western Blot analyses; expression (erbB-2/HER-2 antibody) and activation (phospho-erbB-2/HER-2 antibody) of *neu* were measured. Data were expressed as the ratio between phospho-*neu* over *neu* using actin levels for normalization. Mix caused a 2- to 4-fold increase of the phospho-*neu*/*neu* ratio at both time points. In another series of experiments, a long term treatment (more than 25 passages) with hexachlorobenzene (HCB), an important environmental contaminant, also caused a 2-fold increase in the ratio. Overall, these observations show that environmental contaminants cause an overactivation of a key tyrosine kinase receptor frequently overexpressed in breast cancer tissue. Downstream events may involve overactivation of proteins, such as Akt and ILK. This work brings biological plausibility to the involvement of environmental contaminants in BCD. (Supported by the Toxic Substances Research Initiative, Health Canada)

1497 EFFECT OF 2, 2', 4, 4', 5, 5'-HEXACHLOROBIPHENYL (PCB-153) ON HEPATOCYTE PROLIFERATION IN MICE DEFICIENT IN THE P50 SUBUNIT OF NF-κB.

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Polychlorinated biphenyls (PCBs) are a group of synthetic chemicals that induce and promote liver tumors in rodents. Our previous studies showed activation of the hepatic transcription factor NF-κB in rats treated with PCBs and an increase in hepatocyte proliferation that is correlated with the NF-κB activation. In this study, the effect of NF-κB activation on hepatocyte proliferation after PCB administration was analyzed in male B6129 mice and mice deficient in the p50 subunit of NF-κB. In a 2-day study, mice received a single i.p. injection of corn oil or PCB-153 (300 μmol/kg) and were euthanized after 48 hours. In a 21-day study, mice received six i.p. injections of corn oil or PCB-153 (100 μmol/kg) and were euthanized 4 days after last injection. Nuclear NF-κB DNA binding activity, measured by electrophoretic mobility shift assay, was present in control wild type mice and increased with PCB-153 in the 2-day study. NF-κB DNA binding activity was less in the p50-/- mice and did not increase with PCB-153. Hepatocyte proliferation was measured in these mice by the 5-bromo-2'-deoxyuridine (BrdU) labeling index. Control wild type mice had the same level of cell proliferation as the control p50-/- mice. Cell proliferation increased in the wild type mice treated with PCB-153 in both studies. PCB-153 did not change the cell proliferation in the p50-/- mice in the 2-day study, but the p50-/- mice had a slight increase in cell proliferation after 21 days. These data indicate that NF-κB is involved in the proliferative changes in response to PCB-153. (Supported by P42 ES 07380)

1498 EFFECTS OF VITAMIN E ON NF-κB DOWNSTREAM EVENTS IN P50-/- MICE TREATED WITH THE PEROXISOME PROLIFERATOR, CIPROFIBRATE.

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Peroxisome proliferators (PPs) are a diverse class of chemicals, including some hypolipidemic drugs and plasticizers, which cause a dramatic increase in the size and number of hepatic peroxisomes in rodents and eventually lead to hepatic tumors. The pathway to tumorigenesis by PPs has not been delineated, but oxidative stress is believed to alter signal transduction pathways thereby leading to cell growth disturbances. Nuclear factor-κB (NF-κB) is a transcription factor activated by reactive oxygen and is involved in cell proliferation and apoptosis. We previously found that the peroxisome proliferator ciprofibrate (CIP) activates NF-κB and that dietary vitamin E is effective in decreasing CIP-induced NF-κB DNA binding. We hypothesized that increased dietary vitamin E reduces CIP-induced oxidative stress and cell proliferation by reducing the activation of NF-κB in mice. Sixteen B6129 female mice and twenty homozygous NF-κB1 deficient mice (p50-/-) were fed a purified diet containing 10 or 250 ppm vitamin E (α-tocopherol acetate) for 28 days. At that time, half of the wild-type and half of the p50-/- mice were placed on the same diet with 0.01% CIP for 10 days. Nuclear extracts were prepared from the livers and the NF-κB DNA binding activity was determined using electrophoretic mobility shift assays. Hepatocyte proliferation was determined immunohistochemically by measuring the 5-bromo-2'-deoxyuridine (BrdU) labeling index. In the wild-type mice, CIP-treatment increased the p65:p50 NF-κB activity and increased cell proliferation, yet neither endpoint was significantly altered by dietary vitamin E levels. In contrast, the p50-/- mice had lower NF-κB activation and higher basal levels of cell proliferation and lower GSH/GSSG. The p50-/- mice fed higher dietary vitamin E and treated with CIP had 60% lower cell proliferation in comparison to the group fed low vitamin E. Thus, the effects of vitamin E on cell growth parameters are not solely through decreased NF-κB activation.

1499 EFFECT OF KUPFFER CELL ACTIVATION ON LIVER FOCAL LESION GROWTH.

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The induction of hepatocellular neoplasia in rodents is a multistep process involving a series of cellular modifications. While most studies have focused on the hepatocyte as the target cell, recently a role for other liver cell types, specifically the Kupffer cell, have been suggested to participate in the carcinogenesis process. The purpose of the present study was to investigate the effect of Kupffer cell activation on preneoplastic focal lesion growth in liver. Hepatocellular foci were induced in F344 male rats using diethylnitrosamine. Following focal lesion formation, selected rats were treated with bacterial lipopolysaccharide (0.25 mg/kg I.P. weekly). Rats were sampled after 7 and 28 days of treatment, and livers were examined for DNA synthesis, apoptosis, and mitosis in normal and focal liver lesions. In addition, the relative area and number of hepatic focal lesions was quantified using stereologic methods in H and E and glutathione S-transferase (GST-P) stained histologic sections. Lipopolysaccharide treatment caused a significant increase in GST-P positively stained foci area as compared to control. In addition, a 2-fold increase in relative focal lesion volume (GST-P and H and E) were seen in lipopolysaccharide treated rat liver compared to control. This increase appeared to be mainly localized to eosinophillic foci. While lipopolysaccharide produced an increase in focal lesion size no increase in the number of focal lesions was seen. Lipopolysaccharide treatment also produced an increase in DNA synthesis in both normal liver and focal lesions, although the increase was markedly greater in focal hepatocytes. From these results, it appears that Kupffer cell activation by lipopolysaccharide functions at the promotion stage of the multistage carcinogenesis process.

1500 MECHANISMS OF BUTOXYETHANOL CARCINOGENESIS IN THE RAT AND MOUSE.

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Butoxyethanol induces an increase in liver tumors (hemangiosarcomas) following chronic treatment in the mouse but not in the rat. Concomitant with tumor development is the induction of hemolysis and an increase in hemosiderin deposition in the liver. Previous studies by our group have shown an increase in oxidative damage in mouse liver following butoxyethanol treatment. These observations have led to our proposal that butoxyethanol induces tumors indirectly through activation of Kupffer cells (*via* iron loading) that in turn produces reactive oxygen species as well as the release of cytokines resulting in induction of cell proliferation in the target cells. The present studies examined whether butoxyethanol induced oxidative stress and altered cell growth in the susceptible species (B6C3F1 mouse) and in the nonsuceptible species (F344 rat) following treatment with 0, 225, 450 and 900 mg/kg butoxyethanol for 7, 14, 28, and 90 days. Butoxyethanol produced a bi-phasic response in the mouse. Butoxyethanol (450 and 900 mg/kg) resulted in an increase in oxidative damage in DNA (OH8DG) and lipid (malonaldehyde) at both the 7 and 90 day treatment points. Hepatic vitamin E levels were also decreased at these time points. DNA synthesis was increased in liver endothelial cells at 7 days while DNA synthesis in hepatocytes was only increased at 90 days. No increases in oxidative damage or DNA synthesis were seen in the rat at any dose or time point examined. No changes in apoptosis or mitosis were observed in the liver of either species at any dose or time point. In summary butoxyethanol caused an increase in oxidative damage and DNA synthesis in the mouse (susceptible species) in cell types that correspond to the observed tumors, while no changes were seen in the rat (non-sensitive species). These results support our proposal that butoxyethanol-induced neoplasia occurs through the induction of oxidative stress resulting in an increase in DNA synthesis in the target cell types in the mouse liver.

1501 MECHANISMS OF MACROPHAGE ACTIVATION BY PEROXISOME PROLIFERATORS.

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Although the peroxisome proliferator receptor α(PPARα), localized exclusively in hepatocytes, is required for peroxisome proliferator-induced cell proliferation, evidence has accumulated that Kupffer cell-derived oxidants and mitogenic cytokines are also required in this mechanism. However, it is unknown how peroxisome proliferators activate Kupffer cells. Since circulating Wy-14, 643 is almost exclusively bound to albumin *in vivo*, it was hypothesized that Wy-14, 643 is recognized by macrophage scavenger receptors which function physiologically to recognize modified lipoproteins and albumin. Indeed, the data presented here show that Wy-14, 643 bound to albumin leads to a significant but transient increase in intracellular calcium in a murine macrophage cell line (RAW 264.7 cells); this effect was nearly completely prevented by preincubation with the scavenger receptor inhibitor polyinosinic acid. Interestingly, Wy-14, 643 also lead to a rapid increase in ERK1/2 expression suggesting a role for the MAP kinase pathway in addition to calcium-dependent signaling in mediating macrophage activation in response to peroxisome proliferators. Collectively, these data begin to address for the first time how peroxisome proliferators initially activate Kupffer cells to ultimately result in oxidant and mitogenic cytokine production.

1502 *IN UTERO* ORIGINS OF CANCER IN p53 KNOCKOUT MICE: MATERNAL DIETARY VITAMIN E, FETAL OXIDATIVE DNA DAMAGE AND POSTNATAL CARCINOGENESIS.

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Reactive oxygen species (ROS), including hydroxyl radicals, can oxidatively damage cellular macromolecules such as lipids, proteins and DNA. ROS-mediated oxidative stress and macromolecular damage have been implicated in cancer initiation

and promotion, and the embryo has limited protective antioxidative mechanisms, leaving it at potentially greater risk. We have previously shown that maternal supplementation with the antioxidant vitamin E (VE) alters levels of endogenous embryonic DNA oxidation. Here, to determine whether cancer can have *in utero* origins, we examined the effect of maternal dietary VE (dl-α-tocopherol-acetate) supplementation on spontaneous postnatal tumorigenesis, using high-dose VE and the cancer-prone p53 knockout mouse model. Virgin heterozygous (+/-) p53-deficient females were placed on either a normal or 10% [w/w] VE-supplemented diet for 4 weeks and mated with +/- p53-deficient males. VE supplementation was stopped on the day of birth. The offspring were p53-genotyped and observed for spontaneous tumor development. Compared to offspring from dams supplemented with control diet, *in utero* exposure to VE enhanced spontaneous tumor formation in both +/- and -/- p53-deficient offspring. At 40 weeks of age, VE-exposed -/- p53 pups had less than half the survival rate of -/- p53 control offspring (22% *vs.* 49%) ($p<0.02$). Among +/- p53 offspring, 78% of the VE offspring were still alive at 40 weeks of age, compared to 92% in the control group ($p<0.04$). This tumorigenic enhancement is consistent with a study in CD-1 mice in which high-dose VE failed to reduce endogenous fetal DNA oxidation, and shows that high-dose VE can exacerbate rather than block some ROS-mediated pathologies. These preliminary results indicate that some cancers originate *in utero* and can be substantially modified by altering embryonic redox status, suggesting novel carcinogenic mechanisms and potential therapeutic strategies. (Support: NCIC; CIHR)

1503 PUTATIVE COLON CANCER RISK FACTORS SPECIFICALLY INDUCE DAMAGE OF *P53* IN PRIMARY HUMAN COLON CELLS.

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Introduction: Colon cancers are strongly linked to dietary factors. Although it has been possible to identify whole foods as increasing risks it awaits clarification which individual compounds are involved at the molecular level. One group of candidates includes α, β-unsaturated aldehydes (products of lipid peroxidation) and reactive oxygen species (result from oxidative stress). Both are elevated in tissues and body fluids during unfavourable diets. **Aim:** It is of high interest to experimentally assess their potency for inducing cancer-relevant endpoints in colon cells, and thus to obtain more evidence for their relative impacts during colon carcinogenesis. For this we have developed a novel application utilising fluorescense *in situ* hybridisation of isolated DNA ("comet" structures, single cell microgelelectrophoresis) with *p53* specific probes (Comet FISH). **Methods:** Human epithelial colon cells were isolated from surgical tissue by enzyme digestion. Test compounds were trans-2-hexenal (400-1600μM) and H_2O_2 (18.75-150μM). Global DNA damage was determined with the alkaline comet assay and specific breakage of *p53* with Comet FISH. **Results:** Both compounds induced DNA damage in primary human colon cells. Increased global DNA damage is associated with a higher probability of *p53* breakage. The highest doses induced similar degrees of damage with similar staining patterns for *p53* migration. **Conclusions:** The results clearly show that trans-2-hexenal and H_2O_2 induce genetic damage in primary human colon cells. Moreover, for the first time it has been shown that they induce specific genetic damage in *p53*. The novel and relevant method of Comet FISH is now being developed to compare *p53* breakage with damage in *APC* and *K-ras*. The approach of measuring genotoxic impact in the cancer relevant genes of human colon cells offers new unique possibilities to study potential risk factors and learn more on the aetiology of colon cancer.

1504 MODULATION OF BENZO(A)PYRENE-INDUCED P53 BY ACROLEIN.

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Acrolein, a highly electrophilic α, β-unsaturated aldehyde, is by far the most reactive amongst the aldehydes present in smoke. Levels of acrolein in ambient air are estimated at 0.04 to 0.08 ppm, although it is found at up to 90 ppm in cigarette smoke. Subchronic-inhalation animal studies have reported physiological effects at 0.4 ppm. The relative contribution of acrolein to complex mixture toxicity of smoke at the molecular level remains unknown. Our past work with exposure of human type II (A549) lung cells to acrolein has shown that it inhibits the redox sensitive transcription factors NF-κB and AP-1. We have extended this to study the ability of acrolein to modulate the effect of benzo(a)pyrene (BaP) on p53 protein. The hypothesis being investigated is that a thiol imbalance induced by acrolein can alter the activity of p53 protein induced by BaP. Exposure of A549 cells to 1 μM BaP for 48 h strongly activated the expression of p53 as seen by western blotting, and its DNA binding as shown by electrophoretic mobility shift assay. Treatment of A549 cells with a non lethal dose of acrolein alone (50 fmol/cell for 0.5 h) depleted 80% of total cellular glutathione but did not alter p53 protein levels. When BaP-treated cells (48 h) were exposed to acrolein for 0.5 h there was no effect on BaP-induced p53 protein levels, but a 90% inhibition in the DNA binding of p53 activated by BaP. Using a p53 dual luciferase reporter assay, acrolein caused an 83% decrease in the p53 activity induced by BaP (1 μM for 24 h post transfection). The downstream consequence of inhibiting p53 activity with acrolein is being investigated. Results from this study suggest that acrolein can alter p53 DNA binding and function which may play an important role in lung cancer attributed to cigarette smoke carcinogens such as BaP. (This work was supported by the Maryland Cigarette Restitution fund, NIEHS Center grant ES03819 and RO1 ES09791.)

1505 FOMESAFEN: A MURINE HEPATOCARCINOGEN THAT ACTS *VIA* PPARα TO SUPPRESS APOPTOSIS AND INDUCE CELL AND PEROXISOME PROLIFERATION.

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Fomesafen is a herbicide that increased liver tumor incidence in male and female CD-1 mice when administered in the diet at 100 and 1000 ppm. Previous studies indicated that fomesafen belongs to the peroxisome proliferator (PP) class of non-genotoxic hepatocarcinogens. In rodent liver, PPs activate the peroxisome proliferator activated receptor α (PPARα) causing peroxisome proliferation, suppression of apoptosis and cell proliferation, ultimately leading to tumors. In contrast, human liver is refractory to the adverse effects of PPs. To confirm that fomesafen belongs to the PP class of non-genotoxic hepatocarcinogens, fomesafen was administered to CD-1 mice for 28 days. Fomesafen at 100 or 1000ppm increased mean relative liver weight in male and female mice (1.4- and 1.8-fold and 1.1- and 1.6-fold, respectively, relative to control), and caused a marked induction of hepatic peroxisomal volume in both male and female mice (8.2- and 12.0-fold and 9.4- and 12.5-fold, respectively, relative to control). To assess the ability of fomesafen to regulate cell proliferation and apoptosis, and to assess the role played by PPARα, hepatocytes isolated from control and PPARα null mice were used for *in vitro* investigations. In wild type mouse hepatocytes, fomesafen (250μM) caused peroxisome proliferation, increased the rate of cell proliferation (from 7.6±0.7% to 12.0±1.5% of cells in S-phase) and decreased the rate of apoptosis (from 1.4±0.3% to 0.7±0.1% of hepatocytes). In contrast, PPARα null mice hepatocytes were refractory to the effects of fomesafen on peroxisome proliferation, cell proliferation and apoptosis. Since human liver is resistant to the effects of PPs, the response of human hepatocytes to fomesafen was investigated *in vitro*. Human hepatocytes were refractory to the effects of fomesafen on peroxisome proliferation, cell proliferation and apoptosis. These data demonstrate that fomesafen belongs to the PP class of rodent specific hepatocarcinogens that induce tumors *via* a mode of action that is not relevant for man.

1506 INHIBITION OF APOPTOSIS IN RAT HEPATOCYTES BY 'NON-DIOXIN-LIKE' POLYCHLORINATED BIPHENYLS.

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Polychlorinated biphenyls (PCBs) lead to a variety of biological effects in experimental models. Among other adverse effects in humans, an increased incidence of liver cancer was observed under high exposure to PCBs. In contrast to 'dioxin-like' PCBs, 'non-dioxin-like' PCBs are inactive or almost inactive as aryl hydrocarbon receptor (AhR) agonists. Although they do not share structural properties with phenobarbital (PB) certain 'non-dioxin-like' PCBs show induction of PB-inducible drug-metabolizing enzymes. Like TCDD and PB, several PCBs are tumor promoters in rat liver. Since the inhibition of apoptosis in pre-neoplastic foci has been demonstrated for the liver tumor promoters TCDD and PB, it may also play a central role in the mechanism of tumor promotion by PCBs. Nevertheless, no data concerning an anti-apoptotic effect of tumor-promoting PCBs in pre-neoplastic rat liver are available. This study investigates the possible relationship between 'PB-type' induction of CYP isozymes and the effect on UV-initiated apoptosis in cultured rat hepatocytes after treatment with 'non-dioxin-like' PCBs. For this purpose, hepatocytes from rat liver were cultured using the collagen sandwich technique and apoptosis was initiated by irradiation with UV light. Addition of the PCBs IUPAC numbers 28, 101 and 187, and of PB 30 min after irradiation, suppressed the UV-induced increase in apoptosis in a concentration-dependent manner, following the rank order of PCB 28 > PCB 101 = PB > PCB 187 for apoptosis-suppressing potencies. PB and all PCBs tested also led to a concentration-dependent induction of CYP2B1/2B2 activity following the rank order PCB 187 > PCB 28 > PCB 101 > PB for inducing potencies. For the first time, our results demonstrate the suppression of UV-initiated apoptosis in rat hepatocytes by 'non-dioxin-like' PCBs.

Furthermore, both the inhibition of apoptosis and the 'PB-type' induction of CYP activity show distinct concentration-response relationships, indicating different signaling pathways for both effects.

1507 ROLE OF CHOLINE DEPLETION IN DIETHANOLAMINE INDUCED DNA SYNTHESIS IN MOUSE HEPATOCYTES.

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Diethanolamine increased the incidence and multiplicity of liver tumors in the mouse but not in the rat following chronic dermal exposure. While the mechanism(s) for tumor induction is not readily evident, diethanolamine inhibits cellular choline uptake. Choline deficiency is known to produce tumors in rodents, therefore, one might postulate that diethanolamine, through depletion of choline, may result in the development of tumors in the mouse. The present studies examined the effect of diethanolamine and choline depletion on DNA synthesis in primary cultured mouse hepatocytes. Mouse hepatocytes were cultured with 0 – 500 mg/ml diethanolamine for 24 hours and DNA synthesis measured immunohistochemically, based on BrdU incorporation into DNA. In mouse hepatocytes DNA synthesis was increased following treatment with 10 mg/ml diethanolamine and higher and ranged from 1.8- to 3.2-fold over control. Incubation of mouse hepatocytes in medium containing reduced choline concentrations (1/10 to 1/100 of normal medium; 0.898 mg/L to 0.0898 *vs.* 8.98 mg/L) for 24 hours resulted in an increase in DNA synthesis (1.4- to 2.4-fold over control); whereas incubation of cells in medium containing 10-fold higher choline concentration produced a 50% reduction in DNA synthesis from control. These results showing that reduction of cellular choline results in an increase in DNA synthesis suggest that the DNA synthesis seen following diethanolamine treatment may be a result of cellular choline depletion and may contribute to diethanolamine carcinogenicity.

1508 ENDOTHELIAL-CELL KINETICS IN RAT MODEL OF RIDDELLIINE-INDUCED HEMANGIOSARCOMA.

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Riddelliine is a naturally occurring pyrrolizidine alkaloid nominated by the FDA for carcinogenicity testing because of its economic impact on the livestock industry and the potential for human exposure. In NTP 2-year studies with riddelliine administered by gavage, high incidences of hemangioma and hemangiosarcoma were induced in the liver of rats and mice. In an effort to understand the pathogenesis of hemangiosarcoma, the capacity of riddelliine to damage endothelial cells and induce endothelial-cell proliferation was tested. 42 male Fisher rats received riddelliine (0, 1, and 2.5 mg/kg/day) by gavage for up to 6 weeks. 7 animals/group were sacrificed after 8 consecutive daily doses, and the remainder was sacrificed after 30 doses. By light microscopy, non-parenchymal-cell hypertrophy and karyomegaly were noted in the high-dose livers of the terminal sacrifice. That these cells were immunopositive for Factor VIII suggested them to be endothelial. A slight trend of increase in apoptosis (TUNEL method) was noted in the liver of the high-dose animals of the interim sacrifice. A significant increase in non-parencymal-cell proliferation (BrdU method) was noted in the liver of animals of the interim and terminal sacrifices. A significant increase in parencymal-cell proliferation was noted in the liver of the interim-sacrificed, while an opposite effect was noted in the terminally sacrificed. Electron microscopic evaluation indicated treatment-related damage as well as hyperplasia of non-parenchymal cells. The data suggest that the endothelial cells are specifically damaged by riddelliine. This event may lead to endothelial-cell proliferation, perhaps of the regenerative type. Possible growth factors involved in the proliferation of the endothelial cells are currently being investigated.

1509 15-DEOXY-¡â^{12,14}-PROSTAGLANDIN J_2, A LIGAND OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTER-¥ã INDUCED APOPTOSIS THROUGH G2/M PHASE ARREST IN NEUROBLASTOMA CELLS.

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15-Deoxy-¥Ä15-Deoxy-¥Ä^{12,14}-prostaglandin J_2 (15-deoxy-PGJ_2), a peroxisome proliferator-activated receptor (PPAR-¥ã) ligand, has been shown to stimulate differentiation and induced apoptosis in several cancer cells including breast, prostate and lung cancer cells. In this study, we examined whether 15-deoxy-PGJ_2 could inhibit cell growth through induction of apoptosis in neuroblastoma cells (SK-N-MC and SK-N-SH). We also investigated the expression of (anti-) apoptosis-related genes and activation of transcription factors. 15-Deoxy-PGJ_2 inhibited neuroblastoma cells growth and induced apoptosis in a dose (2-16 ¥M) and time-dependent manner. Consistent with the induction of apotosis, 15-deoxy-PGJ_2 reduced the expression of anti-apoptotic Bcl-2 but increased the expression of pro-apoptotic Bax, caspase 3 and caspase 9. Flow cytometric analysis showed that these cells were arrested in G2/M phase after 15-deoxy-PGJ_2 treatment. Furthermore, 15-deoxy-PGJ_2 significantly increased the expression of cyclin B1, but decreased the expression of cdk4, cyclin D1, cdk2 and cdc2. It was also found that PPAR-¥ã was expressed by 15-deoxy-PGJ_2 in these cells. Taken together, these results suggest that 15-deoxy-PGJ_2 may be a candidate for a preventive or a therapeutic agent for neuroblastoma.

1510 INHIBITION OF ULTRAVIOLET B-MEDIATED ACTIVATION OF NUCLEAR FACTOR KB BY GREEN TEA CONSTITUENT (-)-EPIGALLOCATECHIN-3-GALLATE IN CULTURED HUMAN EPIDERMAL KERATINOCYTES.

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Ultraviolet (UV)-B radiation to mammalian skin results in oxidative stress *via* an increased generation of reactive oxygen intermediates (ROIs). ROIs are known to cause a variety of adverse effects including cancer. Nuclear factor kappa B (NF-kB), a redox sensitive transcriptional factor, plays an important role in regulating the expression of various genes that participate in many physiological processes such as inflammation, apoptosis, and cellular proliferation. Green tea polyphenol (-)-epigallocatechin-3-gallate (EGCG) has been shown to possess photochemopreventive properties. Because, NF-kB has been implicated in UV response, we investigated its involvement in photochemopreventive effects of EGCG. In dose- and time- dependent studies, we evaluated the effect of EGCG on the phosphorylation and degradation of IkB alpha and activation of IKK alpha and NF-kB following UVB irradiation of normal human epidermal keratinocytes (NHEK). Immunoblot analysis demonstrated that the treatment of NHEK with EGCG (10-40 micro M) for 24 hrs before UVB (40 mJ/cm2) exposure dose-dependently inhibited UVB-mediated degradation and phosphorylation of IkB alpha and activation of IKK alpha protein expression. Employing immunoblot analysis and enzyme linked immunosorbent assay, we found that EGCG inhibits the UVB-mediated activation of NF-kB/p65 in the nucleus. In time-dependent study we observed that EGCG (10 micro M) inhibits degradation and phosphorylation of IkB alpha and activation of IKK alpha and NF-kB/p65 at 1, 2, 3, 6, 12 hrs post UVB-exposure. We next examined the effect of UVB on upstream targets of NF-kB. Following UVB exposure, the expression of RANK (receptor activator of NF-kB) and NIK (NF-kB-inducing kinase) proteins remained unaltered suggesting that RANK and NIK are not involved in UVB-mediated activation of NF-kB. These results provide molecular basis for the photochemopreventive effect of EGCG and suggest that green tea may be a useful agent against UVB-induced effect in human skin.

1511 MODULATION OF TRANSFORMED AND NORMAL SYRIAN HAMSTER EMBRYO (SHE) CELL GROWTH BY COMPONENTS OF GREEN AND BLACK TEA.

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The field of chemoprevention has seen increased interest in the last decade. While a number of chemicals have been shown to delay or prevent the cancer process, less is known about the mechanisms by which chemopreventive agents function in this regard. Chemicals can inhibit a tumorigenic growth by function directly on preneoplastic, initiated cells or indirectly on normal cells surrounding them. Using beno(a)pyrene (BP) induced transformed SHE cells and untreated, normal SHE cells, we studied the effects of a component of green tea, EGCG, and of black tea, tea pigment, on the growth and death of preneoplastic and normal SHE cells. Following treatment both EGCG and tea pigment increased the clonal growth rate of normal SHE cells in a dose dependent manner. In contrast, a decrease of clonal growth of the BP transformed cells was observed. Further investigation, showed that the decreased clonal growth of the BP induced preneoplastic SHE cells was through an increase in cell death (apoptosis). These data suggest a selective effect on cell growth and death of the tea components on the preneoplastic and normal SHE cells. This effect was dose responsive in both cell types. No difference was seen at low doses of the components and at high doses, the tea components decreased the growth of both normal and preneoplastic cells. In addition, an increase in DNA damage (using COMET analysis) was seen at high doses of the tea components in both cell types suggesting a risk to promote the growth of transformed cells. These dose-related modifications growth regulation of the tea components in gene expres-

sion of cell proliferation and apoptosis pathways was also examined. These results show a selective effect of tea components on growth in normal and preneoplastic cells that is dose dependent.

1512 INDUCTION OF QUINONE REDUCTASE ACTIVITY IN HEPA 1C1C7 CELLS AND IN FISHER 344 RATS BY BROCCOLI DEPENDS ON SULFORAPHANE BUT NOT ON GLUCORAPHANIN CONTENT.

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Glucosinolates (GS) and their bioactive hydrolysis products such as sulforaphane (SF) upregulates detoxification enzymes and are suggested to be responsible for the chemopreventative effects of cruciferous vegetables. Broccoli (brassica oleracea L., Italica) is the primary source of GS in the American diet. We previously analyzed >50 broccoli varieties and found that the total GS content and individual GS species varied greatly depending on variety. In this study nine broccoli varieties were tested for their ability to increase quinone reductase (QR) activity, a biomarker for anti-carcinogenesis, in mouse hepatoma (Hepa 1c1c7) cells. The objective was to study whether QR activity correlates with total amount of GS, the specific GS glucoraphanin (GP, precursor to SF) or any of the hydrolysis products such as SF. 130, 000 cells/well were plated in 12 well plates 24 h prior to exposure to aqueous extracts of 100, 500 or 1000 mug freeze-dried broccoli/ml medium, for 24 h. The increase of QR activity was dose-dependent and significant differences in QR inducibility among the broccoli varieties were detected: Peto #7 > Ma191 = Peto #15 > Packman = Majestic = Brigadier> VI-158 = EV 6-1. QR inducibility did not correlate with total GS or GP, but did correlate significantly ($p<0.01$) with SF content of the extracts. Two broccoli varieties, Packman and Majestic, were fed to male Fisher 344 rats for 5 days (20% of diet). As in cell culture, hepatic QR induction reflected relative SF levels, but not total GS or GP levels. Results indicate that SF is primarily responsible for increases of QR activity both *in vitro* and *in vivo* and that different broccoli varieties may provide differing chemopreventative potency. Supported by USDA grant # 99-35503-7010

1513 *IN VITRO* TWO-STAGE MORPHOLOGICAL TRANSFORMATION OF SYRIAN HAMSTER EMBRYO (SHE) CELL.

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Cell transformation systems have been used for many years to study the mechanisms of carcinogenesis *in vitro* and to detect the carcinogenic potential of chemicals. Cell transformation to a malignant state is a multistage process, and in SHE cells the first stage is characterized by a morphological transformation (MT). MT may itself be susceptible to a generalized two-stage process of initiation and promotion, as that occurs *in vivo*. Although two-stage transformation models have been demonstrated in several cell transformation systems and exploratory work has been performed with SHE cells, a well-defined method for detecting initiating/promoting activity in SHE cell cultures has not been developed. The initial results of a program to establish an *in vitro* two-stage SHE cell morphological transformation assay system are reported. BaP and TPA were used as an initiator and promoter, respectively. A preliminary study was conducted to determine subtransforming doses of BaP and TPA. No significant increases in the frequency of MT were observed in SHE cell cultures treated with BaP for 24 hours at < 334 ng/mL. Treatment with TPA for 7 days at < 1 ng/mL also did not induce significant increases in the MT frequency. When SHE cell cultures were treated with 334 ng/mL BaP for 24 hours, followed by TPA at 1 ng/mL for 6 days, a MT frequency of 2.18% was observed, compared to 0.63% by BaP treatment alone and 0.56% by TPA alone. Thus, a synergistic effect appeared to occur between the two subtransforming treatments. Similarly, a synergistic effect on MT was also observed in SHE cell cultures treated with BaP at a transforming dose of 5 µg/mL for 24 hour, followed by a subtransforming dose of TPA (1 ng/mL) for 6 days. The MT frequency induced by the sequential treatment was 2.98%, with a frequency of 1.79% for BaP alone and 0.29% for TPA alone. These initial data suggest that a two-stage morphological transformation model can be established using cultured SHE cells and studies designed to fully characterize this model system are in progress.

1514 DEVELOPMENT OF TUMOR PROMOTER TESTING MODEL WITH TRANSFECTED CELL LINES HARBORING C-HA-RASV12G.

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Chemicals can be identified as tumor promoters by their ability to induce tumor in promotion process. To develop a rapid tumor promoter testing system, human mutant c-Ha-rasV12G gene was introduced into human embryo lung fibroblast line WI 38 and human bronchial epithelial cell line BEAS-2B by electroporation. Transfectants were selected with G418 at different concentrations. A series of G418-resistant (G418r) monocolonies from transfected cells were isolated. Following exposure to different concentrations of phorbol-12-Myristate-13 Acetaet(PMA), phenotypes of neoplastic transformation of G418r cells were studied. According to our data, G418r WI 38 cells underwent neoplastic transformation by PMA treatment. Relative coefficiency was 0.9972. No obvious changes were found in untransfected cells. Results about BEAS-2B cells showed: (1) On untransfected cells, when the concentration of PMA was below 100nM, there was no evident growth inhibitory and promoting tumor effects. PMA at concentration over 100nM could induce squamous differentiation ($p<0.05$);(2) Contrastly, G418r cells could be promoted by PMA at concentration below 500nM($P<0.05$), and the treatment of 100nM of PMA for 24h might be mitogenic best. When the concentration of PMA reached 1uM, there would be growth arrest; (3) PMA could endow G418r cells with proliferation reaction induced by fetal bovine serum conditionally. Initiated cells are resistant to terminal differentiation effects of some tumor promoters. Thus, with the development of neoplastic transformation, the resistance to terminal differentiation induced by promoter increases until the promoter becomes mitogenic. The irreversible cessation of division of human epithelial cells expressing a mutant ras oncogene could be induced by PMA at high concentration(>1uM), and the promoting action of PMA may be mainly mediated *via* the activation PKC pathway. Collectively, the two transfected cell lines provide powerful and convenient models for detecting potential tumor promoters and research on mechanism of promotion tumor process.

1515 THE ABSENCE OF FASL ENHANCES THE DEVELOPMENT OF TUMORS AND REDUCES LIFE EXPECTANCY OF P53+/- MICE.

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In the multi-step process of tumor development, several genetic and epigenetic events occur to transform cells from normal to malignant. Although p53 is one of the most commonly mutated genes in a wide variety of tumors, how other genes interact with p53 to transform cells is only just beginning to be understood. To study the effects of the interaction of the Fas system with p53 in tumor progression and development, mice with a targeted disruption of the p53 tumor supressor gene and deficient in Fas ligand were evaluated. Organ weights, life expectancy, and tumor and tissue histology were assessed. Although the absence of FasL had no effect on life expectancy of homozygous p53-deficient mice, the absence of a functional FasL had a profound effect on the life expectancy of p53 heterozygous mice, reducing the median time to death from 12.1 months to 9.6 months. Additionally, the tumor spectrum in p53 heterozygous mice shifted from predominantly sarcomas (63%) when FasL was present to a large number of lymphomas (76%) in FasL-/-p53+/- mice. The reduction in life span and increased incidence of lymphoma in FasL-/-p53+/- mice suggests that these mice could be used in carcinogenicity testing, particularly in understanding the mechanisms of compounds that induce lymphomas. Future studies will examine the possibility of using FasL-/-p53+/- mice as an alternative model in carcinogenicity assessment.

1516 EVALUATION OF THE Tg.AC TRANSGENIC MOUSE FOR PREDICTING THE PHOTOCARCINOGENIC POTENTIAL OF PHARMACEUTICALS: DOSE-RESPONSE OF Tg.AC MICE TO SOLAR-SIMULATED LIGHT.

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Tg.AC mice [FVB/N-Tg(v-Ha-ras)] have been shown to develop large numbers of papillomas within 26 weeks in response to dermal application of certain carcinogens. This propensity to develop skin tumors may make Tg.AC mice an ideal alternative short-term model for photocarcinogenicity testing. Since UV light alone is a carcinogen, our first objective was to determine the threshold levels of solar simulated light (SSL) that are tumorigenic to Tg.AC mice. The acute minimal erythemal dose (Med.) was determined in FVB/N mice (the parental strain). Groups of 7 male and female Tg.AC mice were then exposed to a fraction of the Med. of SSL (0, 0.1, 0.2, 0.3, 0.4, or 0.6 Med.). A similar SSL dose-response study was performed on FVB/N mice to confirm an enhanced tumorigenic response in Tg.AC mice. Mice were exposed 5 days per week for 26 weeks, then observed for an additional 12 weeks with no SSL exposure. No significant papilloma incidence was observed with any group after 26 weeks of treatment with SSL or after 12 additional weeks of observation. Most Tg.AC mice that received the higher SSL doses developed large nonpapilloma skin tumors (4/6 male and 5/5 female in 0.6 Med. group vs 0/4 male and 0/6 female in no SSL group), but only during the 12 week post SSL dosing period of the study. In FVB/N mice, only the female 0.6 Med. group (2/7) developed skin tumors (both nonpapilloma) during the final week (week 38). Unlike typical carcinogen-induced tumors in Tg.AC mice, *in situ* hybridization indicated, surprisingly, that the transgene was not expressed in these UV-induced tumors and immunohistochemical analysis revealed that all tumors were positive for p53 expression. The absence of skin tumors in all SSL treatment groups at 26 weeks indicates

that Tg.AC mice are not highly sensitive to chronic suberythemal SSL exposure and that the "background" incidence will be low when evaluating the Tg.AC mouse response to photocarcinogens in future studies.

1517 COMPARISON OF THE SKH1 AND K6/ODC MOUSE STRAINS FOR SENSITIVITY TO PHOTOCARCINOGENESIS.

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As a result of overexpression of the enzyme ornithine decarboxylase (ODC) in skin, the K6/ODC transgenic mouse is exquisitively sensitive to tumor induction by a wide variety of genotoxic chemicals. In order to determine this model's sensitivity to UV-induced carcinogenesis, K6/ODC mice and the widely used photcarcinogenesis model, SKH1, were subjected to a side-by-side comparison experiment. Groups of mice were exposed to 4 dose levels of primarily UV B radiation from the same bank of Q-Panel lamps on the same days of the week. Mice were irradiated three times a week for 24 weeks. The highest dose (100 mJ/cm^2 3 times per week) corresponded to a weekly dose of 5 minimal erythema doses (MEDs). At the highest dose, K6/ODC and SKH1 mice developed an equivalent tumor incidence (75%) and multiplicity (1.3 tumors/ mouse). At progressively lower doses, however, K6/ODC mice developed more tumors than SKH1. At 60 mJ/cm^2 thrice weekly, the tumor incidence was 45% *vs.* 15% and multiplicity was 0.45 *vs.* 0.15 for K6/ODC and SKH1 mice, respectively. At the two lowest doses (30 and 15 mJ/cm^2 thrice weekly), SKH1 mice developed no tumors while K6/ODC mice had 5 tumors in these dose groups. There were no tumors in unirradiated control groups of either strain. The majority of the tumors were squamous papillomas in both strains, but some squamous cell carcinomas were also present. These results demonstrate the utility of the K6/ODC model for photocarcinogenesis studies, especially at low doses and relatively short exposure periods. Because of it's high sensitivity to both genotoxic chemicals and UV irradiation, the K6/ODC mouse may be uniquely suited for certain applications, such as studies of the interaction of environmental chemicals and UV. (Supported by SBIR grant R43 CA 76901 from NCI, NIH, DHHS)

1518 HISTOPATHOLOGIC EVALUATION OF CLARA CELL-DERIVED LUNG TUMOR PROGRESSION IN A TRANSGENIC MOUSE MODEL.

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The purpose of the present work was to characterize the histomorphological progression and phenotype of Clara cell-derived lung tumors in a transgenic mouse. In this model, the Clara cell secretory protein (CC10) promoter was used to direct expression of the SV40 large T antigen (SV40TAg) to Clara cells. These mice developed lung tumors within 3 months. Early lesions observed at 1 month included terminal bronchioli lined by hyperplastic intensely basophilic cuboidal epithelial cells, which were disorganized and formed small papillary projections. Bronchiolar epithelium was also extending into adjacent alveoli. In larger airways there were multifocal segments of basophilic disorganized cuboidal epithelium interspersed between segments of normal ciliated epithelium. At this time, CC10 expression was markedly reduced as determined by Western blotting and immunohistochemistry. Lung lesions advanced with time, and at 2 months there was a more prominent extension of proliferating epithelium into alveoli. Solid masses of cuboidal cells were forming in terminal bronchioli and papillary lesions were present in medium to larger bronchioles. By 3 months, solid tumor masses were starting to compress adjacent alveoli and there were focal areas of disorganized small anaplastic cells. At 4 months, tumor size increased with obvious compression of adjacent alveoli and there was evidence of pleural fibrosis and pleural lymphatic invasion. This transgenic mouse model, characterized by extensive bronchiolar hyperplasia which progresses rapidly to tumor formation, provides new insight into changes associated with Clara cell transformation and differentiation. The model also demonstrates the early loss of CC10 expression in Clara cell-derived tumors.

1519 DOXYCYCLINE (DOX)-REGULATED, LUNG-SPECIFIC EXPRESSION OF MUTANT HUMAN Ki-*ras* IN BITRANSGENIC MICE: ALTERATIONS IN LUNG MORPHOLOGY.

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Mutation in Ki-*ras* is a critical and early event in the development of lung adenocarcinomas in both humans and animal tumor models. Development of transgenic mice that possess constitutively active Ki-*ras* has been attempted but was unsuccessful because expression of the transgene resulted in embryonic lethality. We have thus developed a bitransgenic mouse model that allows for the regulation of Ki-*ras* expression in a lung-specific and DOX-inducible manner. A CYS^{12} mutant allele of the human Ki-*ras* gene was linked to a tetracycline (tet)-inducible promoter and then used to establish a line of transgenic mice with this construct. These mice, which do not express the Ki-*ras* gene, were then crossed with a second trangenic line containing the reverse tet activator (rtTA) expressed constitutively from either the SPC or CC10 lung-specific promoters. Induction of the Ki-*ras* transgene was detected by RT-PCR in bitransgenic mice following 1 week of DOX exposure. DOX-treated bitransgenic mice exhibited hyperplastic lung foci after only 12 days of DOX-treatment. By 5 weeks of treatment, extensive epithelial hyperplasia of the alveolar region of the lung tissue could be seen. Lung morphology in untreated bitrangenic mice or either of the DOX-treated single transgenic or control mice was normal. The hyperplasia noted in the lungs is consistent with preneoplastic lesions observed in chemically-induced mouse lung tissues that precede the onset of frank neoplasia. These results demonstrate that the bitransgenic mouse model will provide an important new research tool that will allow us to determine the role of Ki-*ras* mutations in lung tumorigenesis. This will have important implications for future research on the etiology, genetics, prevention, and development of novel therapies for lung cancer.

1520 REPLACEMENT OF PARTIAL HEPATECTOMY WITH FASTING/REFEEDING OR THE USE OF NEONATAL ANIMALS IN THE RESISTANT HEPATOCYTE MODEL.

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In rat liver several models have been developed to study multistage carcinogenesis, including the Solt-Farber resistant hepatocyte model. In this model initiation consists of either a necrogenic dose of a hepatocarcinogen or a non-necrogenic dose in conjunction with partial hepatectomy. As an alternative to partial hepatectomy, we investigated two different procedures: fasting/refeeding or the use of one-day-old neonates. The experimental design consisted of four groups: 1) control; 2) fasted for 4 days, then refed; 3) partial hepatectomy; 4) neonates. Groups 1-3 were injected p.o. with diethylnitrosamine (DEN) (100mg/kg) 24 hrs after refeeding or partial hepatectomy. Group 4 was treated with the same dose of DEN at one day of age. All initiation animals were treated with selection agents, 3 daily doses of 2-acetylaminofluorene (2-AAF), followed by a single dose of carbon tetrachloride(2ml/kg) followed by 3 additional daily treatments of 2-AAF. Rats were killed two weeks after the last treatment. Most of the rats in the partial hepatectomy and fasting/refeeding groups developed visible nodules. All three experimental groups developed significantly more gamma-glutamyl transpeptidase (GGT)- positive foci per cu cm of liver tissue than in the control group. In the fasting/refeeding group, the size and the volume % of GGT- positive foci in liver tissues were higher compared to rats subjected to partial hepatectomy. Furthermore, the number of GGT-positive foci in neonates was higher compared to the partial hepatectomy group. We conclude that both fasting/refeeding and the use of neonatal rats can replace partial hepatectomy as a method of initiation in the Solt-Farber method since both of these methods appear to be less stressful to the animals and equally sensitive. (Supported by ES 07380)

1521 ALTERED LEVELS OF PITITUARY AND SEX HORMONES IN MEXICAN AGRICULTURAL WORKERS EXPOSED TO ORGANOPHOSPHATE PESTICIDES.

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Many pesticides are considered endocrine disruptors due to their capacity to block or activate steroid hormone receptors and/or to affect reproductive hormone levels in experimental models. However, information regarding organophosphate pesticide (OP) exposure and their effects on humans is limited. The objective of this study was to evaluate the relationships between pituitary and sex hormone levels and OP exposure. A longitudinal study was conducted in an agricultural community located in north Mexico where OP were the most widely sold and used pesticides. Hormone levels were measured according to WHO. OP exposure was evaluated by measuring urinary levels of six dialkyl phosphates and phosphorothioates and by an exposure index constructed on the basis of frequency and intensity of OP exposure. FSH serum levels were negatively associated with OP metabolites DMTP and DMDTP. FSH levels were also significantly decreased in the most exposed group. LH serum levels were also negatively associated with DMTP and DMDTP. Marginal decreases in prolactin levels were also observed. Regarding sexual hormones, significant increases in estradiol serum levels were observed in the periods of higher exposure and they were positively associated with DMP and DETP urinary concentrations. Testosterone levels increased during the period of highest exposure and were positively associated with DMTP concentrations. Our results strongly suggest that high OP exposure alters the homeostatic relationships between the pi-

tuitary and the testes. Further studies are needed to determine the specific targets responsible for the effects here reported. (Partially supported by CONACyT (Grant No. 28403-M), WHO (Grant No. 96349) and by The Advanced Training Program of The Border XXI Initiative.

1522 NEUROBEHAVIORAL EVALUATION OF RESIDUAL EFFECTS OF LOW-LEVEL BYSTANDER ORGANOPHOSPHATE PESTICIDE EXPOSURE.

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Organophosphate pesticides (OP's) may cause neurotoxicity at low-levels of exposure. Subject: A female, 42 years old, high school graduate, farmer, working in a hay field, wearing shorts and a swim suit top, without respiratory protection was exposed to Thimet and possibly Lorsban applied to a downwind neighboring field in 6/98, with re-exposure by working in the adjacent field 6-8 times over the next 3 days. The evening of the exposure, she experienced headache, nausea, vomiting, leg pain, diarrhea, chest tightness, dizziness, blurred vision, and weakness in legs and arms - symptoms of OP poisoning - with continuing vertigo, nausea, pain etc.. Later and persistent symptoms included memory loss, sleep disorder, fatigue, irritability, and impaired executive function. ACHE testing was inconclusive. Assessment: Included comprehensive neurobehavioral toxicity evaluations, 2 years post-exposure, with an extensive interview and testing, and record review (educational, medical, letters of reference). Findings: Prior IQ was at the 80%tile. The Neurotoxicity Screening Survey showed results consistent with those of patients diagnosed with neurotoxicity. Current Full-scale IQ had declined to the 47%tile, with Processing Speed (a factor very sensitive to global neurotoxicity) at the 8%tile. Additional deficits were seen in detecting visual figure-ground relationships - 36%tile; Selective Reminding Test (measures learning) <1%tile; Stroop Color and Word Test (evaluates mental flexibility) 31%tile; Visual Search and Attention Test 23%tile; logical memory <1%tile; with moderate anxiety and moderate-severe depression. Distortion was below the level of detection. Malingering was ruled out by 5 separate tests. Personality testing using the NEO Personality Inventory found no personality disorders that could contribute to the findings. Record review found no competing explanations of her illness. Conclusion: Relatively low-level organophosphate exposure can cause neurotoxicity, revealed by neurobehavioral evaluation, lasting many years after exposure.

1523 AN APPROACH FOR SCREENING CHOLINESTERASE INHIBITORS IN DRINKING WATER.

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Under the 1996 amended Safe Drinking Water Act (SDWA), a Contaminant Candidates List (CCL) has been compiled in 1998 (FR 63:40). Several Cholinesterase (ChE) inhibitors were identified in this list, some are organophosphates and others are carbamates. Prior to 1996, ChE inhibitors in drinking water were regulated individually and monitored by using conventional analytical methods to detect the presence and concentration of individual contaminants. Because of specific advances in analytical chemistry and the need for cumulative risk assessment of chemicals that have a common mechanism of toxicity, we developed an approach for initially screening for the presence of ChE inhibitors in drinking water. This approach is based on the use of a Microwell Plate Assay kit. In this method, Acetyl Cholinesterase (AChE) was stabilized in a gelatin film to determine low levels of pesticide contaminants in drinking water that inhibit ChE activity. The remarkable properties of the dry immobilized ChE preparation include its stability to prolonged storage at room temperature as well as its stability to short term elevated temperatures (60 degrees C). The enzyme could be maintained in dry gel form for 365 days at room temperature without substantial loss of activity. Several procedures were evaluated to oxidize less potent organothionophosphate (P=S) compounds to their more inhibitory oxon forms. Inhibition profiles were run for six commonly used carbamate insecticides (and some of their metabolites) and eight organophosphate insecticides using this assay. IC_{50} and IC_{20} values were determined for purified water and several drinking water matrices. Results using this assay were also compared to a commercially available test kit. This method may be successfully used for screening drinking water ChE inhibitors individually or in mixture. The opinions expressed are those of the authors and do not necessarily reflect USEPA policy.

1524 ACETYLCHOLINESTERASE INHIBITION: PREDICTION USING THREE-DIMENSIONAL QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS.

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Organophosphorus pesticides are known to produce their biological effects through the inhibition of a number of esterases, including acetylcholinesterase (AChE), the enzyme responsible for the degradation of the neurotransmitter acetylcholine. The combined use of conformational analysis and three dimensional (3D), quantitative structure-activity relationship (QSAR) methods were used to rationalize the inhibitory potencies of a series of organosphorus pesticides against AChE. The CATALYST program was used to identify the structural features in the group of eight inhibitors whose IC_{50} values ranged from 0.34 nM to 1.2 µM. The 3-D pharmacophore models were characterized by at least one hydrogen bond acceptor site and 2-3 hydrophobic sites and demonstrated good correlation (r^2=0.994) between the predicted and experimental IC_{50} values. This approach can be used to screen databases of organophosphorous and other chemicals for their neurotoxic potential *via* the inhibition of AChE.

1525 ASSESSING THE IMPACT OF HUMAN PON1 POLYMORPHISMS: SENSITIVITY AND MONTE CARLO ANALYSES USING A PHYSIOLOGICALLY BASED PHARMACOKINETIC/PHARMACODYNAMIC (PBPK/PD) MODEL FOR CHLORPYRIFOS.

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A PBPK/PD model was developed for chlorpyrifos (CPF) and the active metabolite CPF-oxon. Susceptibility to organophosphate (OP) insecticides is associated with variation in pharmacokinetic/ pharmacodynamic response. A genetic polymorphism in the PON1 (arylesterase) detoxification of OPs results in the expression of a range of PON1 enzyme activities within humans. The objective was to identify sensitive parameters that influence the model output, and to investigate the impact of human PON1 status on CPF-oxon brain concentration over a range (5 µg/kg - 5 mg/kg) of CPF doses. The model was used for parameter sensitivity analysis, and Monte Carlo simulations for PON1 activity utilizing the human PON1 polymorphic distributions. Based on the sensitivity coefficients (SC), the model was sensitive to the following parameters: CPF-oxon plasma protein-binding, liver partitioning, liver PON1 metabolism, plasma butyrylcholinesterase (BuChE) and blood flow to the liver and brain. In addition, the SC for plasma protein binding, PON1, and BuChE demonstrated a clear dose-dependency. The model simulations suggest a dose-dependent non-linear increase in the brain CPF-oxon AUC, at doses >0.5 mg/kg, which is a function of both dose and PON1 activity. In contrast, at low environmentally relevant doses (~5 µg/kg) the model is relatively insensitive to the variability in PON1 activity. The results suggest that other esterase detoxification pathways may adequately compensate for lower PON1 activity; hence an increased sensitivity to low PON1 is not observable until non-target esterases have been appreciably depleted. This response is consistent with simulations that show an increased sensitivity to PON1 status at doses that significantly deplete plasma BuChE activity in humans (>90% inhibited). This study illustrates the utility of PBPK/PD modeling for determining the overall impact of parameter uncertainty and variability on risk assessment predictions for OP insecticides. (Supported by EPA grant R828608)

1526 EFFECTS OF DIETARY CHLORPYRIFOS ON PERIPHERAL TISSUE *VERSUS* BRAIN AND RBC ACETYLCHOLINESTERASE ACTIVITY IN DOGS.

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Chlorpyrifos (CPF) is a widely-used broad-spectrum insecticide. At the request of the United Kingdom's Advisory Committee on Pesticides, a 6-week dog study was conducted to examine the effects of dietary exposure to CPF on peripheral tissue (left atrium, nodose ganglia, diaphragm, and quadriceps muscle) acetylcholinesterase (AChE) activity as compared to AChE in brain and red blood cells (RBC). Six-month old Beagle dogs (n = 4/sex/dose) were given 0, 0.5, 1.0, or 2.0 mg/kg/day CPF in their diets. AChE was assayed using acetylthiocholine in the presence of the butyrylcholinesterase inhibitor iso-OMPA (0.1 mM). AChE activities were compared using Dunnett's test (alpha = 0.05). RBC AChE showed a slight (~10%) but statistically significant decrease in the mid- and high-dose groups after 1 day of exposure to CPF. RBC AChE was inhibited at all dose levels after one week, reaching a steady-state of inhibition within three weeks of exposure. After 6 weeks of treatment at 2.0 mg/kg/day, a small difference in brain AChE (~7% inhibition; males and females combined) may represent a true treatment effect but was not statistically significant and was not considered adverse (<20% inhibition). Peripheral tissue AChE activity was corrected for connective tissue content as AChE activity in diaphragm, quadriceps, and nodose ganglia was inversely-correlated with the amount of connective tissue in the sample (r^2 < 0.49). No statistically significant differences in peripheral tissue AChE were found. However, the left atrium AChE activity in high-dose males was about 25% lower than controls, and may represent a true treatment effect despite a comparable but opposite effect in females, which indicated random variability. We conclude that RBC AChE is more

sensitive than peripheral tissue or brain to inhibition by CPF. The acute RBC NOAEL was 2.0 mg/kg and the repeated-dose NOAEL for brain and peripheral tissue was at least 1.0 mg/kg/day.

1527 METHODS TO EVALUATE PERIPHERAL TISSUE ACETYLCHOLINESTERASE ACTIVITY IN DOGS TREATED WITH CHLORPYRIFOS.

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Total cholinesterase activity (ChE), as measured by reaction with acetylthiocholine (ASCh), is often used as an estimate of acetylcholinesterase activity (AChE) in animals treated with anticholinesterases. Measurements of ChE may overestimate the potential effects of anticholinesterases on AChE by ignoring the contribution of tissue butyrylcholinesterase (BuChE), for which ASCh is also a substrate. Moreover, the relative content of AChE and BuChE in peripheral tissues is not fully understood in many species, including dogs. Therefore, methods were optimized to measure tissue AChE activity in dogs treated with the anticholinesterase chlorpyrifos (CPF). ChE activity was evaluated in the RBC, brain, nodose ganglion, left atrium, left ventricle, diaphragm muscle, and quadriceps muscle of male Beagle dogs (8-9 months old). Dogs (3/dose level) were treated with CPF at 0, 0.3, 0.6 or 1.2 mg/kg/day in their diets for 4 weeks. ChE and BuChE were measured by reaction with ASCh or butyrylthiocholine, respectively. AChE activity was isolated from ChE using iso-OMPA, a specific inhibitor of BuChE. Brain ChE activity was largely (92%) composed of AChE. The percent of ASCh activity attributed to AChE in peripheral tissues ranged from 40% (left ventricle) to 87% (nodose ganglion). Overall, RBC AChE and tissue BuChE were inhibited at all dose levels. There was no apparent inhibition of brain or peripheral tissue AChE (ASCh with iso-OMPA). Small differences in tissue ChE (ASCh without iso-OMPA) were attributed to inhibition of BuChE. We conclude that RBC AChE is more sensitive than brain or peripheral tissue AChE, and that the importance of considering the contribution of BuChE when assessing AChE activity cannot be overstated.

1528 *IN VIVO* DETOXICATION OF ORGANOPHOSPHATES BY HEPATIC AND SERUM ESTERASES.

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The protective effect(s) were studied of hepatic and serum A-esterases (AEST), which catalytically hydrolyze organophosphate (OP) oxons, and carboxylesterases (CaE), which stoichiometrically degrade OP's, for brain cholinesterase (ChE) during *in vivo* exposures to chlorpyrifos-oxon (CPO) and paraoxon (PO) at postnatal days (PND) 3 and 80. Rats were injected ip with either corn oil or the specific CaE inhibitor, bis-(4-nitrophenyl) phenylphosphonate (BNPP), followed by injections of CPO or PO. OP dosages were as follows for PND3 and 80, respectively: CPO (0.5 and 15 mg/kg), and PO (0.1 and 1.5 mg/kg). At PND3, brain ChE activity was inhibited 76% and 81%, in male rats exposed to BNPP followed by exposure to CPO or PO, as compared to 44 and 17% for CPO or PO treatment only, respectively. At PND3, activities were similar for males and females. At PND80, ChE inhibition was 84% and 95% in males exposed to BNPP-CPO or BNPP-PO, respectively. However, activity was similar to corn oil control levels for PO exposure, but was 50% inhibited after CPO exposure. For PND80 females, brain ChE inhibition was 88% and >90%, in rats exposed to BNPP-CPO and BNPP-PO, respectively, and 57% and 70% inhibited for CPO or PO exposures. The protective effect of CaE can be shown in the difference between ChE activities in the presence and absence of CaE. To speculate on AEST protection *in vivo*, and using *in vitro* inhibition data, it is possible to calculate the effective OP concentration required for the observed % inhibition *in vivo*, and to compare this to the dosage (in micromoles/kg) administered to BNPP-treated animals. For PND3 males, these calculated values [in ratios of nM/(micromoles/kg)] were 4.9 for PO and 5.9 for CPO. For PND80 males, which have much higher AEST levels, the values were 3.6 for PO and 0.28 for CPO. These data suggest that AEST are more important for CPO hydrolysis, whereas CaE are more effective in detoxication of both CPO and PO. (Supported by NIH F31 ES05752).

1529 INHIBITION OF PARAOXONASE (PON1) BY HEAVY METALS.

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Paraoxonase (PON1) is a serum enzyme that hydrolyzes multiple substrates, including oxidized lipids, lactones, and organophosphorus (OP) compounds such as the nerve agents sarin and soman and the oxon forms of the insecticides parathion, diazinon, and chlorpyrifos. PON1 activity is determined in part by the Q192R coding region polymorphism, which affects its catalytic activity, and in part by differences in PON1 expression levels, which can vary as much as 13-fold among individuals of the same genotype. Because PON1 plays an important role in protecting against environmentally-induced disease, as well as atherosclerosis, we sought to determine the effects of heavy metals on PON1 activity and expression. In an *in vitro* assay of PON1 activity, cadmium, mercury, zinc, and iron, at levels less than 1 μM, inhibited the activity of purified human PON1 by more than 90%. Inhibition by iron, but not the other metals, was specific to the $PON1_{192R}$ isoform, with less striking effects on $PON1_{192Q}$. Despite this robust inhibition of PON1 activity by heavy metals *in vitro*, chronic exposure of mice to cadmium chloride (up to 0.5 mg/kg daily for two weeks, resulting in blood cadmium levels as high as 1 μM) did not decrease serum PON1 activity significantly. Similarly, chronic exposure of mice to methylmercury hydroxide (up to 7.5 mg/kg daily for two weeks) did not decrease PON1 activity significantly in either serum or liver microsomes. We conclude that environmental exposure to either cadmium or methylmercury is not likely to affect PON1 activity. Metal-binding proteins in the blood seem to be sufficient to protect PON1 against inhibition by these metals. Supported in part by ES 04696, ES 07033.

1530 DEVELOPMENT OF AN *IN VITRO* ASSAY WHICH MAY IDENTIFY WHICH ORGANOPHOSPHORUS PESTICIDES ARE MORE TOXIC TO THE YOUNG.

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Some, but not all, organophosphorus pesticides are more acutely toxic to the young as compared to adults. We have developed an *in vitro* assay which measures the detoxification potential (*via* carboxylesterase and A-esterases) of tissues. Previous results using this *in vitro* assay correlated with the marked *in vivo* sensitivity of the young to chlorpyrifos, and also correlated with the equal sensitivity of the young and adult to methamidophos (Padilla *et al.*, Neurotoxicology, 2000). We have now extended these observations to two other pesticides that have already been shown in the literature to be more toxic to the young: paraoxon and malathion. In our *in vitro* assay, liver and plasma from 7-day old rats were much less effacacious than adult tissues at detoxification of the active metabolites of these two pesticides-a pattern that correlates with the *in vivo* sensitivity of the young to those two pesticides. Using our *in vitro* assay we also tested the active metabolite of diazinon, diazoxon, and again found that young liver or plasma possessed much less detoxification capability than adult tissues. From these results, we predicted that young animals would be more sensitive to diazinon, which, in fact, was the case: when postnatal day (PND)17 or adult rats were given a dosage of 75 mg/kg diazinon, adult brain cholinesterase (ChE)actvity was only inhibited 38%, while brain ChE in the PND 17 animals showed much more inhibiton (75%). Furthermore, higher doses (200-500 mg/kg) were lethal to PND17 rats, but adults only showed signs of toxicity. We conclude that our *in vitro* test may prove to be a useful, quick convenient method for predicting *in vivo* age-related sensitivity to organphosphorus pesticides. *This abstract does not reflect EPA policy.*

1531 LACK OF SEX DIFFERENCES IN ACETYLCHOLINESTERASE INHIBITION IN NEONATAL RAT FOREBRAIN FOLLOWING ACUTE AND REPEATED ORAL EXPOSURE TO CHLORPYRIFOS.

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Chlorpyrifos (CPS), one of the most widely used organophosphorus insecticides in the USA, exerts its toxicity through the inhibition of acetylcholinesterase (AChE). Multiple studies have reported a marked susceptibility of young animals to the toxic effects of CPS compared to adult animals, which has evoked increased concerns about its safety. The literature has reported significant sex differences in the inhibition of AChE following acute exposure to CPS in neonatal rats. In this study, postnatal day 1 (PND1) male and female rat pups were acutely exposed by oral gavage to 1.5 or 3.0 mg/kg CPS. AChE activity was determined in the forebrain at various times following exposure to determine the time of peak inhibition of AChE to identify any possible differences between sexes. Following 1.5 mg/kg CPS, peak inhibition (57-59%) occurred at 12 hours with minor recovery of activity by 24 hours (47-50%). Following 3.0 mg/kg, peak inhibition (87%) occurred at 3-4 hours with little recovery by 12 hours but significant recovery by 24 hours (51-56%). Time to peak inhibition and inhibition levels were very similar for both males and females suggesting that following an acute oral exposure, male and female neonates respond similarly. In addition, male and female rat pups were repeatedly exposed from PND1-PND6 by oral gavage to these dosages of CPS. The re-

duction of forebrain AChE activity was similar between males and females on PND 4, 7, and 12 with inhibition levels of 25-29%, 31-33%, and 12-13% for 1.5 mg/kg CPS and 41-46%, 44-48%, and 18% for 3.0 mg/kg, respectively. These data suggest that following repeated CPS exposure, male and female rat pups also respond similarly.

1532 AGE- AND TIME-DEPENDENT EFFECTS OF EQUITOXIC DOSAGES OF PARATHION OR CHLORPYRIFOS ON HIGH AFFINITY CHOLINE UPTAKE IN RAT CORTEX AND STRIATUM.

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High affinity choline uptake (HACU) in brain is altered in adult rats after exposure to some organophosphorus (OP) toxicants. Immature rats are more sensitive than adults to the toxicity of OP insecticides parathion and chlorpyrifos, but little is known regarding age-related effects on HACU. AChE and HACU in cortex and striatum of neonatal (7 days), juvenile (21 days) and adult (90 days) rats (n=8-15/treatment group) were evaluated at 4, 24 or 96 hr after equitoxic exposures to parathion (LD10 = 0.8, 1.5 and 6.6 mg/kg, po) or chlorpyrifos (LD10 = 15, 47 and 136 mg/kg, po). HACU was studied by incubation of synaptosomes with [3H]choline and subsequent separation of incorporated and unincorporated label by rapid filtration. Parathion elicited relatively similar levels of peak ChE inhibition in all age groups (64-82% at 4 hrs after dosing). Chlorpyrifos also elicited similar age-related reductions in ChE activity (81-91%), but peak inhibition was earlier in neonates and juveniles (4 hrs) than in adults (24 hrs). HACU was increased after parathion exposure, with maximal increases occurring at 4 hrs after exposure in neonatal striatum (52%), juvenile cortex (19%) and striatum (23%), and at 96 hrs after treatment in adult cortex (41%) and striatum (36%). In contrast, HACU was generally reduced by chlorpyrifos, with maximum reduction at 4 hrs after dosing in neonates (43%), 24 hrs in juveniles (22%) and 96 hrs in adults (18%). Interestingly, HACU was increased (15%) in neonatal striatum at 96 hrs after chlorpyrifos. Increased uptake of choline could lead to higher acetylcholine synthesis and amplification of the toxic consequences of parathion-induced acetylcholinesterase inhibition. Alternatively, a reduction in HACU following chlorpyrifos could potentially limit the amount of ACh released into the synapse and thereby reduce cholinergic toxicity. Changes in HACU may contribute to OP toxicant-selective and age-related toxicity following AChE inhibition by these two insecticides. (Supported by STAR grant GR825811 from USEPA).

1533 COMPARATIVE TOXICITY OF ORAL CHLORPYRIFOS EXPOSURES IN ADULT AND AGED RATS.

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Chlorpyrifos (CPF) is a widely used organophosphorus insecticide that elicits toxicity through inhibition of acetylcholinesterase. A number of studies have reported that CPF is more toxic to immature animals and that aged rats (15 and 24 months of age) are not differentially sensitive to subcutaneously administered CPF. We evaluated the relative sensitivity in adult (3 months) and aged (15 months) rats to oral CPF exposures. Adult (3 months of age) and aged (15 months of age) Sprague-Dawley rats (n=5) were challenged with an effective but sublethal dosage (LD1) previously determined in adult rats (i.e., 80 mg/kg, po in peanut oil). Adult rats showed no signs of functional toxicity while aged rats exhibited moderate to severe signs (principally autonomic) and 2/5 lethality. Additional groups (n = 5) were treated daily with CPF (0, 1, 3 or 10 mg/kg, po) for 14 days and sacrificed for neurochemical measurements 4 hrs after the final dosing. None of the adult or aged rats exhibited functional signs of toxicity throughout the dosing period. Blood cholinesterase inhibition was greater in aged rats (71%) than in adults (55%) at 1 mg/kg/day group. Diaphragm ChE was significantly increased (16-25%) in adult rats at 1 and 3 mg/kg/day while in aged rats there was a dose-related reduction in activity (15-66%). In frontal cortex, ChE inhibition was significantly greater in aged rats (81%) compared to adults (39%) at 3 mg/kg/day, whereas at 10 mg/kg/day, ChE inhibition was similar in both groups (86-92%). These results suggest that aged rats may be more sensitive than adults to both acute and repeated oral CPF exposure. (Supported by grant ES09119 from NIEHS).

1534 CHRONIC DIETARY EXPOSURE WITH INTERMITTENT SPIKE DOSES OF CHLORPYRIFOS FAILS TO ALTER FLASH- OR PATTERN REVERSAL-EVOKED POTENTIALS IN RATS.

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Human exposure to pesticides is often characterized by chronic low level exposure with intermittent spiked higher exposures. Visual disturbances are often reported following exposure to xenobiotics, and cholinesterase-inhibiting compounds have been reported to alter visual function. This study examined the effects of chronic (1 year) dietary exposure (0, 1, or 5 mg/kg/day) to chlorpyrifos in male Long Evans rats (100-110 days old at study initiation). These doses were chosen to produce minimal and approximately 50% inhibition of brain cholinesterase activity, respectively. In addition to dietary exposure, half of the animals received an oral bolus of 45 mg/kg chlorpyrifos (in corn oil) every other month (n = 16-18 rats/treatment). Subjects were weight maintained at 350g throughout the study. After the final spiked exposure, the animals were allowed to recover for about 2.5 months, so only irreversible effects would be examined. Subjects were surgically implanted with screw electrodes over the visual cortex and allowed to recover for one week. Unanesthetized animals were placed in a restrainer and presented with vertical bar stimuli (square wave spatial and temporal modulation) of 0.06, 0.13, and 0.26 cpd visual angle, at contrast levels of 15, 30, and 60 percent contrast. Reversal rate was 0.49 Hz, and a mean illuminance of 50 lux was maintained between stimuli. After pattern testing, the animals were presented with a 10 μs flash stimulus at 11, 39, and 148 lux-s, with an ambient illumination of 15 lux. Dietary exposure to chlorpyrifos (alone or in combination with oral spike doses) did not produce changes in either flash- or pattern reversal-evoked potentials. The evoked potentials did show the expected intensity and spatial frequency dependent changes, showing that the animal's responses were under stimulus control. Thus, chronic exposure to chlorpyrifos did not appear to alter visual responses at the level of the cortex in adult animals. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

1535 PATTERN OF CHOLINESTERASE INHIBITION IN ADULT, MALE RATS CHRONICALLY EXPOSED TO DIETARY CHLORPYRIFOS.

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Very little is known about the effects of chronic exposure to relatively low levels of anticholinesterase insecticides or how the effects of chronic exposure compare to higher, intermittent exposure of the same compound for the same duration. To that end, we exposed adult male rats to the anticholinesterase insecticide, chlorpyrifos (CPF)for one year at three levels of dietary exposure: 0, 1, or 5 mg/kg/day. In addition, those groups also received a bolus dosage of CPF (*i.e.*, "spiked" animals; 60 mg/kg initially and 45 mg/kg thereafter) in corn oil every two months; therefore, there were 6 dosage groups, n=5/group. Animal tissues were taken for analysis during dosing (at 6 or 12 months) and also 3 months after dosing ceased (*i.e.* "recovery" animals). Tissues analyzed were retina, whole blood and components, diaphragm, and central nervous system (pons). In general, the groups receiving dietary CPF exposure only showed substantial cholinesterase (ChE) inhibition (80%) in the blood at 1 mg/kg/day and no inhibition in retina, pons, or diaphragm; the higher dosage (5 mg/kg) produced more blood inhibition and approximately 50% or less ChE inhibition in the other tissues. One day after receiving the oral, spike dose, all three feeding (*i.e.*, 0, 1 or 5 mg/kg/day) groups showed more than 70% inhibition in each tissue. In the dietary exposure groups (unspiked), the ChE inhibition at 6 months was, in general, not different from the inhibition at 12 months, indicating that a steady state had been reached prior to 6 months. Three months after CPF dosing ended, ChE levels had returned to control levels in all groups. These data indicate that although chronic feeding with or without intermittent spiked dosages with CPF produces substantial ChE inhibition in a dose- and tissue-related manner, ChE activity recovers to normal within 3 months after dosing is ceased. *This abstract does not reflect EPA policy.*

1536 NEUROBEHAVIORAL EVALUATION OF RATS EXPOSED TO CHLORPYRIFOS *VIA* CHRONIC DIETARY AND REPEATED HIGH-LEVEL SPIKE EXPOSURE.

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This study aimed to model long-term subtoxic human exposure to an organophosphorus pesticide, chlorpyrifos (CPF), and to examine the influence of that exposure on the response to intermittent high-dose acute challenges. Adult Long-Evans male rats were maintained at 350g body weight by limited access to CPF-laced food pellets to produce an intake of 0, 1, or 5 mg/kg/day (n=40/feed group). During the year-long exposure, half of the rats in each feed group received bi-monthly challenges (spikes) of CPF, and the other half received vehicle. Rats were periodically tested, using a neurobehavioral screening battery and motor activity, to evaluate the magnitude of the acute response (spike days) as well as recovery and ongoing chronic effects (non-spike days). In non-spiked rats, the lower feed level produced blood but no brain cholinesterase inhibition, whereas the higher feed level produced about 50% brain cholinesterase inhibition. Effects of the spikes differed as a function of dietary level for several endpoints (*e.g.*, tremor, lacrimation), and in

general, the high-dose feed groups showed greater effects of the spike doses. In the intervening months, neurobehavioral differences among treatment groups were influenced more by the spike administration than by the level of CPF in the feed. Thus, chronic and intermittent CPF exposure produced long-lasting neurobehavioral changes and altered the response to acute challenges. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1537 CHOLINERGIC CHANGES AND NEUROBEHAVIORAL DEFICITS FOLLOWING CO-EXPOSURE WITH CHLORPYRIFOS AND NICOTINE IN RATS.

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Approximately 20% of the US population is directly exposed to nicotine *via* direct smoking of tobacco. Many more are exposed indirectly through environmental tobacco smoke. In addition to exposure to nicotine, many of the same individuals are exposed to environmental neurotoxic chemicals such as the organophosphorus pesticide, chlorpyrifos. In the present study, we evaluated the cholinergic system in the central nervous system and neurobehavioral performance following combined exposure with low doses of nicotine and chlorpyrifos. Male Sprague-Dawley rats (250-300 g) were treated daily with nicotine (0.1mg/kg, s.c., in normal saline) or chlorpyrifos (1.0 mg/kg, dermal, in ethanol) or a combination of nicotine and chlorpyrifos for 30 days. Control animals were treated with saline and ethanol. On day 30 sensorimotor functions were evaluated using a battery of tests that included measurements of postural reflexes, limb placing, grip time, beam walking and incline plane performance. The data from behavioral observations showed that there was a significantly exacerbated deficit in incline plane performance, beam-walk score and beam-walk time following combined exposure than exposure with each chemical alone. A significant increase in nAChR ligand binding was observed in brainstem and cortex following exposure with nicotine or chlorpyrifos that was further augmented with combined exposure, while mAChR showed only a modest increase in binding in the cortex. A decrease in cerebellar and an increase in midbrain AChE was observed following combined exposure whereas exposure to nicotine or chrorpyrifos alone did not show any significant change brain regional AChE activity. These data suggest that combined exposure with physiologically relevant doses and route of nicotine and chlorpyrifos may cause additive neurobehavioral deficit and differential changes in the cholinergic system. Supported, in part by an EPA grant # R829399-01-0

1538 PROTECTION AGAINST PARATHION TOXICITY BY SINGLE OR REPEATED ADMINSTRATION OF PHYSOSTIGMINE IN RATS.

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The protective effects of physostigmine against the toxicity of parathion were examined in male Sprague-Dawley rats. Physostigmine (100 or 1, 000 μg/kg, ip) injected 30 min before decreased the inhibition of acetylcholinesterase (AChE) activities in brain, lung and blood induced by parathion (2 mg/kg, ip). Physostigmine pretreatment did not affect the total hepatic microsomal cytochrome P450 (CYP) or cytochrome b_5 contents. There was no change in p-nitrophenol hydroxylase, p-nitroanisole demethylase or aminopyrine N-demethylase activities in liver of physostigmine-pretreated rats. However, erythromycin N-demethylase activity, a selective CYP3A marker, was significantly inhibited by the carbamate in a dose-dependent manner. Physostigmine also decreased erythromycin N-demethylase activity in brain. Physostigmine did not alter carboxylesterase or paraoxonase activity, indicating that detoxication of parathion was not affected by this carbamate. The pharmacokinetic parameters of parathion (3 mg/kg, iv) were determined after a single dose of physostigmine. In physostigmine-pretreated rats, the area under the curve (AUC) of parathion was significantly greater; clearance (Cl) was lower than control rats. Physostigmine (100 or 1, 000 μg/kg/day, ip) was repeatedly administered to rats for 7 consecutive days. When parathion was given to rats 4 or 24 hr after the final dose of the carbamate, the brain AChE inhibition was reduced significantly. Repeated administration of physostigmine did not affect erythromycin N-demethylase activity or the pharmacokinetic parameters of parathion. However, there was a significant decrease in specific [^{3}H] QNB binding in striatum and hippocampus. Scatchard analysis revealed a significant decrease in Bmax without a change in Kd. The results suggest that the inhibition of CYP3A plays an important role in the decrease of parathion toxicity provided by an acute dose of physostigmine, but changes in characteristics of muscarinic receptor could account for the protection against parathion toxicity in rats treated with this carbamate repeatedly.

1539 RELATIVE INHIBITORY POTENCIES OF CHLORPYRIFOS OXON AND CHLORPYRIFOS METHYL OXON FOR HEN BRAIN MICROSOMAL ACETYLCHOLINESTERASE (ACHE) AND NEUROPATHY TARGET ESTERASE (NTE).

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The relative inhibitory potency (RIP) of an organophosphorus (OP) inhibitor against ACHE *versus* NTE may be defined as the ratio, [k_i (ACHE)/k_i (NTE)], where k_i is the bimolecular rate constant of inhibition for a given inhibitor against each enzyme. RIP values > 1 correlate with the inability of OP inhibitors or their parent compounds to produce OP compound-induced delayed neurotoxicity (OPIDN) at doses below the LD50. Chlorpyrifos oxon (CPO) has a RIP value >>1 for enzymes from hen brain homogenate, and the parent compound, chlorpyrifos (CPS), cannot produce OPIDN in hens at sublethal doses. The present study was carried out to test the hypothesis that the methyl homologue of CPO, chlorpyrifos methyl oxon (CPMO), also has a RIP value >>1. Hen brain microsomes were used as the enzyme source and k_i values ($X \pm$ SE) were determined at 37°C for ACHE and NTE (n = 3 and 4 separate experiments, respectively). The k_i values for CPO against ACHE and NTE were 17.8 ± 0.3 and 0.0994 ± 0.0049 μM^{-1} min^{-1}, respectively, yielding a RIP value of 179 ± 9. The k_i values for CPMO against ACHE and NTE were 10.9 ± 0.1 and 0.0582 ± 0.0013 μM^{-1} min^{-1}, respectively, yielding a RIP value of 187 ± 4. The results demonstrate that the RIP values for CPO and CPMO are comparable and both are >>1, suggesting that neither parent compound, CPS nor chlorpyrifos methyl, could produce OPIDN at sublethal doses. (Supported in part by a contract from Dow AgroSciences).

1540 IMMUNOLOGIC DETECTION & AFFINITY SELECTION OF ORGANOPHOSPHATE-ACETYLCHOLINESTERASE ADDUCTS USING ACTIVE SITE SPECIFIC ANTIBODIES.

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Organophosphate (OP) insecticides are rapidly absorbed to quickly infiltrate blood and nervous tissue, resulting in covalent inactivation of acetylcholinesterase (AChE). Exposure to OP compounds, therefore, is expected to correlate with formation of an OP-conjugate as determined by measurement of total erythrocyte and serum ChE activity. This method is both limited by population variation in total ChE levels and the inability to identify the responsible OP compound. It is our hypothesis that OP-AChE conjugates can be reliably detected by antibodies raised specifically against an active site sequence in both its native and phosphorylated states. Such antibodies would be capable of quantifying OP-modified cholinesterase and capable of identifying the OP compound. We have prepared antibodies specific to the active site sequence of mammalian AChE in both its native and phosphorylated states. Western Blots were employed to examine binding of a panel of antibodies against the native and OP-modified enzyme. Antibodies raised against the active site domain of AChE recognize the untreated enzyme but fail to bind to AChE covalently modified by OP's. In contrast, antibodies raised against the phosphorylated active site bind to POCl3 treated AChE (formation of phosphoserine) but do not bind AChE or AChE inactivated by a phosphoester group. To confirm selectivity of the custom antibodies, samples of treated and untreated AChE were trypsinized and resultant peptides isolated by affinity chromatography using the active site antibodies. Peptide samples were further separated by capillary liquid chromatography and sequenced by mass spectrometry. Using a combination of immunologic techniques, synthetic chemistry and mass spectrometry this presentation outlines methods to detect, identify and differentiate OP-AChE adducts.

1541 AUTOANTIBODY DETECTION USING ELISA AND WESTERN BLOT IN DELAYED NEUROPATHY INDUCED BY ORGANOPHOSPHATES (OP).

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Earlier studies demonstrated the attenuating effects of calcium channel blockers, and calpain inhibitors on the development of OP-induced delayed neuropathy (OPIDN) in hens. This modifying effect was evidently due to the attenuation of the calcium-dependent protease calpain's activity in brain and nerves. Selective calpain inhibitors I (CaI) and II (CaII) were used to modify the development of OPIDN following a single dose of phenyl saligenin phosphate (PSP, 3 mg/kg, im,

n=8). CaI (n=8) and CaII (5 mg/kg, im, n=7) were administered immediately before PSP and 24h after PSP. Functional impairment, evaluated by measurement of stride width and length, was greatest in hens treated with PSP only. The amelioration of the degree of OPIDN development by CaI and CaII was confirmed by assay for serum autoantibodies to neurofilaments (NFs), using ELISA and by gel electrophoresis. SDS gels of purified proteins and whole brain homogenates were used for immunoblot, and the protein bands visualized using HRP-conjugated goat anti-chicken IgG following addition of serum dilutions. Autoantibodies to NFs were detected as early as 6 days post-PSP exposure and predominated in hens not receiving calpain inhibitors. SDS gels showed bands with greater immunostaining at 60, 160, and 200 kD for serum from hens treated with PSP only. Anti-NF detection was consistent with the neurodegenerative effects of some OPs. The results confirm earlier studies of autoantibody detection against nervous system proteins in sera of humans and animals exposed to neuropathy-inducing OPs. This data also confirms the possible involvement of calpains in the development of OPIDN, and suggests that calpain inhibitors can modify OPIDN. The detection of serum autoantibodies against nervous system proteins, using ELISA, may be useful in monitoring the development of neuropathy. (Supported by NIH HD35965 and USDE P217A990192).

1542 ORGANOPHOSPHATE PESTICIDES DIFFERENTIALLY ALTER AXONAL AND DENDRITIC GROWTH IN CULTURED SYMPATHETIC NEURONS.

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There is increasing evidence that cognitive and behavioral problems may be linked to perinatal exposure to environmental chemicals, such as organophosphate pesticides (OPs), however, the mechanism(s) by which OPs cause these changes in neuronal function have yet to be determined. We used primary cultures of sympathetic neurons to investigate the possibility that OPs interfere with morphogenic events critical to establishing neuronal connectivity, specifically axonal and dendritic outgrowth. When grown in serum-free medium in the absence of glial cells, sympathetic neurons extend a single axon and no dendrites. In contrast, neurons grown in the presence of either glial cells or bone morphogenetic proteins (BMPs) extend multiple dendrites. Neurons grown under these various conditions were exposed to chlorpyrifos (CPF, 0.01 uM to 10 uM) or its oxon metabolite, CPFO (0.01 nM to 10 nM) for 24 or 72 hr then immunostained for either axon- or dendrite-selective antigens. Axonal and dendritic growth were quantified using Metamorph image analysis software. A 24-hr exposure to either CPF or CPFO causes a significant inhibition of axonal outgrowth in sympathetic neurons even at the lowest concentrations tested. In contrast, a 72-hr exposure to the same concentration range of these OPs weakly induces dendritic growth in the absence of BMPs, and enhances dendritic growth observed in the presence of BMPs. These effects on axonal and dendritic growth occur in the absence of effects on cell viability or acetylcholinesterase enzymatic activity, which indicates that OPs may exert specific and differential effects on axonal and dendritic growth. These data support the hypothesis that OPs disrupt patterns of neuronal connectivity in the developing brain.

1543 REMEDIATION OF ORGANOPHOSPHORUS NEUROTOXICITY IN SY5Y NEUROBLASTOMA CELLS BY ORGANOPHOSPHORUS HYDROLASE (OPH).

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In a previous study, model organophosphorus insecticides (OPs) produced anti-acetylcholinesterase activity, and mipafox and paraoxon modified the expression of the proteins associated with the neuronal cytoskeleton in human SY5Y neuroblastoma cells. The patterns of protein modification were different between mipafox and paraoxon. Numerous approaches have been studied to degrade OP compounds and remediate their toxicity. In the current study, biodegradation of model OP compounds and its efficiency to prevent or reduce neurotoxicity of the compounds were tested with SY5Y cells. OPs preincubated with organophosphorus hydrolase (OPH) did not inhibit acetylcholinesterase (AChE) and neuropathy target esterase (NTE) in SY5Y cells as potently as did buffer-treated OPs. The anti-AChE activity of paraoxon (maximum 3 μM) and anti-NTE activity of mipafox (250 μM) in SY5Y cells were completely prevented by degradation with OPH. Mipafox (40 μM) produced significant modifications in expression of MAP2ab and NF200 proteins in SY5Y cells, while equimolar paraoxon did not. The reaction of NGF-differentiating SY5Y cells to mipafox on protein modulation was dependent on the stage of differentiation at which the OP compound was applied. Neurofilament 200 protein in SY5Y cells exposed to the OPH-treated mipafox containing about 4 μM of mipafox remained after biodegradation was downregulated when given during the early stages of cell differentiation, but upregulated when given at a late stage. Biodegradation of mipafox with OPH significantly reduced neurotoxicological stress on the modulation of these neuroskeletal protein biomarkers. These results support the conclusion that OPH can be used to biodegrade OPs and remediate their neurotoxic effects *in vitro*.

1544 EFFECTS OF NEUROPATHY-INDUCING ORGANOPHOSPHORUS (OP) COMPOUNDS ON MITOCHRONDRIAL ATP PRODUCTION IN PRIMARY DORSAL ROOT GANGLIA CELL CULTURES.

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Cultures of dorsal root ganglia (DRG) can achieve neuronal maturation with axons. They have not, however, previously been used to investigate metabolic events that occur following exposure to neuropathy-inducing organophosphorus (OP) esters. Recent studies in other systems demonstrated *in vivo* alterations of ATP concentrations and changes in mitochondrial transmembrane potential following exposure to neuropathy-inducing OP compounds, suggesting that mitochondrial dysfunction occurs. We report an investigation using chick embryo DRG cultures to evaluate effects on the mitochondrial respiratory chain associated with exposure to these toxicants. This approach uses an *in vitro* neuronal system from the animal species (chicken) that provides the USEPA-approved animal model for OP-induced delayed neuropathy (OPIDN). DRG were obtained from 9-10 day old chick embryos, and grown for 14 days in minimal essential media (MEM) supplemented with bovine and human placental sera and growth factors. Cultures were then treated with 1 uM phenylsaligenin phosphate (PSP), mipafox, paraoxon, or the DMSO vehicle. Mipafox and PSP readily elicit OPIDN in hens; paraoxon does not. *in situ* evaluation of ATP production measured by bioluminescence assay in neurons treated with PSP and mipafox, but not paraoxon, showed significantly ($p<0.05$) decreased ATP concentrations. Values (in pmol/cell, mean ± SEM, n=6) were 307 ± 8 in control cultures, and 271 ± 18, 179 ± 5 and 256 ± 14 in paraoxon, PSP, and mipafox-treated cells, respectively. This low energy state was present in several levels of the mitochondrial respiratory chain, including complexes I, III and IV, although complex I was the most severely affected (<40% of control following mipafox and PSP treatments, $p<0.05$; paraoxon had a nonsignificant effect). We conclude that mitochondria are a primary target for neuropathy-inducing OP compounds, resulting in depletion in ATP concentrations.

1545 PHENYL SALIGENIN PHOSPHATE INDUCED SPINAL CORD GENE EXPRESSION CHANGES IN ADULT *GALLUS GALLUS*.

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Adult hens (*Gallus gallus*) exposed to a single dose of phenyl saligenin phosphate (PSP) develop delayed onset central-peripheral distal axonopathy. Mechanisms of this axonopathy are thought to be initiated by inhibition and aging of the carboxylesterase called neurotoxic esterase (NTE). However, events subsequent to NTE inhibition/aging and onset of neurologic deficits 7-10 days later are poorly understood. We hypothesized that spinal cord gene expression changes occur in this clinically silent period in PSP treated hens, but not in hens treated with the vehicle (dimethyl sulfoxide, DMSO), or phenylmethyl sulfonyl fluoride (PMSF), a compound that results in NTE inhibition without aging and does not result in axonopathy. Spinal cord mRNA transcript expression was evaluated 24 hours after a single im dose of PSP (2 mg/kg), PMSF (90 mg/kg) and DMSO by a cDNA screening approach (targeted display) and Northern analysis on select cytoskeletal genes (vimentin, neurofilament-M, alpha-tubulin). A time course was subsequently undertaken on those genes that showed altered expression at 24 hours. Alpha-tubulin transcript was increased 38% at 12 hours ($p=0.0125$); while at 48 hours PSP and PMSF treated birds had 28% and 42% decreased transcript ($p=0.0576$ and 0.0062, respectively). Two differential expressed genes were identified by targeted display in PSP treated hens. Both showed small (20-30%) but highly significant ($p<0.01$) expression changes at 12 hours. One gene is unidentified, the other is 82% homologous to the human gene KIAA1307. Current work is focusing on identifying the cell/neuronal types that are modulating the expression of these genes.

1546 ORGANOPHOSPHORUS COMPOUND-INDUCED DELAYED NEUROTOXICITY (OPIDN) IN WHITE LEGHORN HENS AS ASSESSED BY HEMATOXYLIN/EOSIN AND FLUORO-JADE STAINING.

K. Carlson, B. S. Jortner and M. Ehrich. *Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.*

Organophosphorus compounds may induce a delayed neuropathy (OPIDN) having one of two patterns, namely Types I and II OPIDN (Abou-Donia and Lapadula, 1990). In this study we evaluated the distribution and extent of lesions of

OPIDN in White Leghorn hens as determined by staining of brain and spinal cord with hematoxylin and eosin (H&E) and a Fluoro-Jade, fluorescent stain for degenerating neurons and their processes. Hens were injected with single dosages of the Type I neurotoxicant phenyl saligenin phosphate (PSP, 2.5 mg/kg im), the Type II neurotoxicant triphenyl phosphite (TPPi, 500 mg/kg sc), or the vehicle dimethyl sulfoxide (DMSO, 0.5 ml/kg, im or sc). They were evaluated daily for clinical signs of neural dysfunction and sacrificed after one, two, and three weeks. PSP- and TPPi-injected hens displayed progressive neurological dysfunction beginning about one week post-administration. The brain and lumbar levels of spinal cord were prepared for microscopic study, as noted above. Samples of the nerve to the biventer cervicis muscle were also prepared for light microscopic examination. H&E stained sections from PSP-dosed hens revealed degenerating myelinated fibers in regions such as long spinal cord and medullary tracts. TPPi-dosed hens had these along with neuronal necrosis in spinal, brainstem and deep cerebellar regions. Staining with Fluoro-Jade revealed more profound and extensive time-dependent PSP- and TPPi-induced degeneration of nerve fibers, terminals, and cell bodies (for TPPi) in spinal, brainstem and cerebellar regions. Both compounds elicited peripheral nerve axonopathy progressing to Wallerian-like degeneration of a similar (severe) intensity. This study demonstrates the utility of Fluoro-Jade staining to reveal the extent of neuronal degeneration in OPIDN, and the presence of a peripheral neuropathy in the Type II form.

1547 A QUANTITATIVE HISTOCHEMISTRY TECHNIQUE FOR MEASURING REGIONAL DISTRIBUTION OF ACETYLCHOLINESTERASE AND BUTYRYCHOLINESTERASE IN THE BRAIN USING DIGITAL SCANNING DENSITOMETRY.

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Studies of brain acetylcholinesterase (AChE) are traditionally based on biochemical assays, immunoreactivity, and histochemistry. Conventional histochemistry yields rich morphological data from tissue sections but yields quantitative results only with great difficulty. Several histochemical methods developed in recent years, including microdensitometry, microphotometry and video-based histochemistry, are effective in quantitative and detailed study of AChE in tissue sections. However, they are usually time-consuming. As we report here, we adapted digital scanning densitometry to quantitate AChE histochemical staining in brain sections. The AChE and butyrylcholinesterase (BuChE), as measured by the method, were heterogeneously distributed throughout the brain, results that are consistent with those obtained by biochemical methods. The staining intensity is dependent on section thickness, substrate concentration, and reaction time. The cholinesterase inhibitor methyl paraoxon significantly decreased AChE staining intensity. Furthermore, data acquired from densitometry are similar to those obtained by video-based microscopy or by spectrophotometry. The advantage of the densitometric measurements compared to other quantitative histochemical methods is that it is very rapid, while collecting data that are equivalent in quality. Because the digital scanning densitometers provide high quality and sensitive imaging, wide dynamic ranges, and convenient image analysis software, they are very useful tools in quantitative histochemistry.

1548 MONITORING BLOOD ACETYLCHOLINESTERASE ACTIVITIES IN RATS GIVEN MULTIPLE DOSES OF ORGANOPHOSPHORUS COMPOUNDS.

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Acetylcholinesterase (AChE) inhibitions in whole blood and brain were used to examine interactions of organophosphorus (OP) compounds in adult male Long-Evans rats given chlorpyrifos (60 mg/kg sc) followed in one week by oral tri-ortho-tolyl phosphate (TOTP, 75, 150 or 300 mg/kg) every other day for 2 weeks before sacrifice. AChE inhibition was monitored in blood 48 hr after dosing with chlorpyrifos, 5 days after initiating TOTP treatment, and on the day of sacrifice. At sacrifice, AChE activities in hippocampus, cortex, basal forebrain and caudate putamen of rats given only chlorpyrifos were 45% ± 5 (mean ± SE, n = 6), 33% ± 5, 43% ± 7, and 39% ± 7 of control, respectively. AChE activities in these brain regions after treatment with chlorpyrifos + 300 mg/kg TOTP were 23% ± 5, 18% ± 2, 25% ± 5, and 18% ± 2, respectively. AChE activities after chlorpyrifos + 300 mg/kg TOTP were in the range of 11 - 18% of control in brain regions from rats drinking corticosterone-treated water (400 ug/ml) beginning 1 week before chlorpyrifos administration. Chlorpyrifos caused blood AChE activities to drop to 45% ± 2 of control 48 hr after dosing with activity remaining this depressed at the time of the second bleeding, but returning to 85% ± 12 of control (considerably higher than in any brain regions) at time of sacrifice. Following chlorpyrifos and 5 days and 2 weeks of 300 mg/kg TOTP, blood AChE activities were 41% ± 11 and 31% ± 7 of control, respectively. Lower doses of TOTP caused less AChE inhibitions in both blood and brain. Corticosterone in drinking water did not alter blood AChE activities. Therefore, blood AChE activities provided dose-response data, although activities were significantly higher than in brain of rats given one OP compound (chlorpyrifos) weeks earlier. (Supported by DAMD 17-99-1-9489. This abstract does not necessarily reflect the position or policy of the US Government.)

1549 BEHAVIORAL CHANGES IN RATS GIVEN TRI-ORTHO-TOLYL PHOSPHATE (TOTP) AFTER CHLORPYRIFOS AND CORTICOSTERONE: LACK OF DOSE-RELATED CORRELATION WITH EFFECTS ON GLUTATHIONE PEROXIDASE, SUPEROXIDE DISMUTASE AND GFAP.

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Motor activity, a Functional Observational Battery (FOB), and passive avoidance were used to evaluate rats given tri-ortho-tolyl phosphate (TOTP) 75, 150 or 300 mg/kg po every other day for 2 weeks, beginning 1 week after a single administration of chlorpyrifos (60 mg/kg sc). In addition, half of the rats in each group were given corticosterone (400 ug/ml) in drinking water beginning 7 days before administration of chlorpyrifos. The behavioral endpoints were measured the day before dosing with chlorpyrifos, and 2 and 3 weeks later. Chlorpyrifos administration resulted in decreased beam breaks (an indicator of motor activity) in the presence and absence of corticosterone (p=0.007). TOTP decreased ambulation after 2 weeks of dosing. Chlorpyrifos exaggerated effects on motor activity and on limb grip strength caused by corticosterone treatment alone. No treatment affected passive avoidance. Biochemical studies at terminal sacrifice revealed that neither corticosterone, TOTP or chlorpyrifos had effects on glutathione peroxidase (GPx) or superoxide dismutase (SOD) activities in the cerebral cortex or hippocampus. Glial fibrillary acidic protein (GFAP) was elevated by all treatments (corticosterone, TOTP, chlorpryfos) in cerebral cortex, but effects were not dependent on dose of TOTP. These results indicate that each treatment had effects on behavior, but they were not dependent on alteration of enzymes indicative of oxidative stress (GPx, SOD). GFAP, however, appeared useful for identification of rats exposed either to corticosterone or organophosphates. (Supported by DAMD 17-99-1-9489. This abstract does not necessarily reflect the position or policy of the US Government.)

1550 THE ORGANOPHOSPHORUS NEUROTOXICANT TRIORTHOTOLYL PHOSPHATE CAUSES LIVER DYSFUNCTION IN RATS.

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The interactions of stress and exposure to neurotoxic chemicals are a matter of considerable interest in toxicology. In a study designed to evaluate these, young adult male Long-Evans rats were administered the following (alone or in various combinations): 400 ug/ml corticosterone in the drinking water (the stress model) from days 1-63;60 mg/kg sc chlorpyrifos on days 7 and 42; tri-otho-tolyl phosphate (TOTP, 75, 150 or 300 mg/kg) by gavage every other day from days 14-26 and 49-61. This portion of the study evaluated clinical chemistry and hematologic findings in the blood collected at sacrifice (day 63). Corticosterone exposure alone or in combination with chlorpyrifos produced no significant variation of analytes. However, a significant alteration in cholesterol concentration (mean 114 mg/dL compared to 85 mg/dL in controls, p = 0.017) occurred with TOTP exposure at 150 mg/kg. Exposure to TOTP at 300 mg/kg produced alterations in cholesterol (mean 125 mg/dL, p = 0.003) and alanine aminotransferase (ALT) activity (mean 142 U/L compared to 52 U/L in controls, p = 0.041). TOTP at 75 and 150 mg/kg combined with corticosterone and chlorpyrifos individually or in combination produced significant alterations in cholesterol concentrations (mean range 127 - 171 mg/dL, p = 0.028 - 0.056). In addition, TOTP at 300 mg/kg, combined as described above, resulted in alteration in albumin concentration (mean 2.8 g/dL p = 0.055), albumin globulin ratio (mean 0.073, p = 0.055), urea nitrogen concentration (mean 13.3 mg/dL, p = 0.001), cholesterol (mean 131 mg/dL, p = 0.030), and ALT activity (mean 153 U/l, p = 0.032). Therefore, multiple administration of TOTP to Long Evans male rats at doses of 75 mg/kg and greater appears to alter hepatocellular membrane permeability and cholesterol metabolism, and TOTP at 300 mg/kg resulted in reduced hepatic function. (Supported by DAMD 17-99-1-9489. This abstract does not necessarily reflect the position or policy of the US Government.)

1551 LOW-LEVEL SOMAN EXPOSURE LEADS TO SYNAPTIC DAMAGE IN THE HIPPOCAMPUS AND POTENTIATES NEURONAL VULNERABILITY.

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Hippocampal slice cultures were used to examine the action of the organophosphate agent soman (GD). Soman is known to inhibit acetylcholinesterase, causing accumulation of ACh, but it also appears to increase glutamate release to promote seizure-related damage. In the present study, the effects of low concentrations of soman on hippocampal tissue were assessed over an extended series of exposures. Single application was used to induce a period of toxicity until agent hydrolysis, and this was repeated daily for one week. No evidence of degeneration was found after the first day of treatment, however, dose-dependent declines in pre- and post-synaptic proteins occurred after a week. The decline in synaptophysin was evident at the 100-nM dose of soman, and further reduction was found at 20 μM (ANOVA: p=0.001, F=9.7). Synaptophysin was reduced throughout the hippocampus, especially in dendritic fields of CA1 and in the molecular layer of the dentate gyrus. Synaptic toxicity appears first since overt cellular pathology was not visible in the three subfields. The decline in the postsynaptic GluR1 subunit was only evident at the 20-μM soman dose ($p<0.001$, F=11.8). Soman also elicited a pronounced increase ($p<0.01$) in spectrin breakdown mediated by calpain, a protease known to cause neurodegeneration in response to glutamate-induced excitotoxicity. Interestingly, while no degeneration was found after the first day of soman treatment, pathogenic changes did occur when the soman insult was combined with a trimethyltin (TMT) insult - TMT is known to cause seizures and hippocampal damage. A 1-h TMT exposure did not cause appreciable synaptic deterioration or spectrin breakdown in control slices, however, robust levels of degeneration were evident when such a TMT insult was followed by a single application of soman. Thus, low-level soman exposure causes synaptic damage in the hippocampus and potentiates vulnerability to other types of toxic insults. Supported by US Army Med.. Res. grant DAMD17-99-C9090, and NIH grant 1R43NS38404-01.

1552 ACUTE EXPOSURE TO SARIN INCREASES BLOOD-BRAIN BARRIER (BBB) PERMEABILITY AND INDUCES NEUROPATHOLOGICAL CHANGES IN THE RAT BRAIN.

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We evaluated the early changes in the adult brain of male Sprague-Dawley rats following single i.m injection of 1, 0.5, 0.1, and 0.01xLD50 of sarin. Twenty four h after treatment, both sarin-and vehicle-treated control animals were processed for: 1) BBB permeability, 2) brain acetylcholiesterase (AChE) and plasma butyrylcholinesterase (BChE) activity, 3) brain m2 muscarinic acetylcholine receptor (m2 mAChR) ligand binding, and 4) histopathological examination of brain. Animals treated with 1 and 0.5 x LD50 exhibited a significant inhibition of BChE activity, while 1 x LD50 caused a significant inhibition of AChE in the cerebrum, brainstem, midbrain, and cerebellum. Ligand binding to m2 mAChR was decreased in cerebrum and showed a significant increase in brainstem. Analysis of [3H]hexamethonium iodide uptake demonstrated a significant increase in BBB permeability in animals treated with1 x LD50. The LD50 dose caused significant decrease in the expression of BBB protein, a diffuse neuronal cell death and a decrease in MAP-2 positive elements within the cerebral cortex and hippocampus, and degeneration of Purkinje cells in the cerebellum. Animals treated with 0.5, 0.1, or 0.01 x LD50 did not exhibit the above changes expect in the cerebellum, where animals treated with 0.5 x LD50 showed Purkinje neuron loss. Thus, the early brain damage after acute exposure to sarin induces significant injury in many regions of the adult brain by 24 h after exposure. The early neuropathological changes observed after a single 1x LD50 sarin could lead to a profound long-term neurodegeneration in many regions of the brain, and neurobehavioral abnormalities. Supported, in part by the US Army Medical Research and Materiel Command under contract DAMD 17-98-C-8027.

1553 LOW-LEVEL SARIN VAPOR EXPOSURE IN RATS: EFFECT OF EXPOSURE CONCENTRATION AND TIME ON PUPIL SIZE.

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Sarin (GB) vapor exposure is associated with both systemic and local toxic effects occurring primarily *via* inhalation and ocular routes, respectively. The objective of this study was to develop a model for predicting dose-response effects (miosis) of GB vapor concentration as a function of exposure duration. Groups of male and female rats (Sprague-Dawley) were exposed to one of a series of GB vapor concentrations for a single duration (10, 60 or 240 minutes), in a whole-body dynamic chamber. Separate effective concentrations for miosis (defined as a post-exposure pupil diameter 50% or less of the pre-exposure pupil diameter) in 50% of the exposed population (EC50) and corresponding dose-response slopes were determined for each exposure duration by the Bliss probit method. In addition, blood esterase activities (acetylcholinesterase, butyrylcholinesterase and carboxylesterase) were measured before and after each exposure. Pupil constriction was the only clinical sign noted, given the GB vapor exposure conditions used in this study. Overall, female rats were more sensitive than male rats to GB vapor-induced miosis. Linear regression analysis was used to derive a model of low-level GB vapor-induced pupil diameter changes in the rat as a function of exposure concentration and time. Contrary to that predicted by Haber's Rule, ECT50 values increased with exposure duration (i.e., the CT for 50% of the exposed population to show miosis was not constant over time). Blood esterase activities were not significantly inhibited by the low-level exposures of GB vapor used in this study.

1554 CHARACTERIZATION OF A *S*-HEXAHYDRO-1*H*-AZEPINE-1-CARBONYL ADDUCT PRODUCED BY MOLINATE ON RAT HEMOGLOBIN β2 AND β3 CHAINS *IN VIVO*.

L. J. Zimmerman, H. S. Valentine, K. Amarnath and W. M. Valentine. *Pathology, Vanderbilt University, Nashville, TN.* Sponsor: W. Valentine.

Molinate is a thiocarbamate herbicide used in the rice industry for over 25 years and has been shown to produce testicular toxicity in male rats. It is has been proposed that bioactivation of molinate *via* sulfoxidation to form the sulfoxide and sulfone metabolites capable of protein binding, may be involved in molinate-induced toxicity. Based on previous *in vitro* studies, carbamylation of an active site serine residue in Hydrolase A has been proposed to be the mechanism responsible for the observed testicular toxicity. The experiments presented here utilize hemoglobin to characterize covalent protein modifications produced *in vivo* by molinate in rats dosed intraperitoneally as a function of exposure duration. Examination of globin from molinate-treated rats by HPLC demonstrated a new peak in the isolated samples that when collected and analyzed using MALDI-TOF MS, revealed a 126 Da increase in mass relative to the native β3 chain. Digestion of the globin using Glu-C and analysis by MALDI-TOF MS revealed two modified peptide fragments at *m/z* 2743 and 4985 consistent with a 126 Da increase to peptide fragments [122-146] and [102-146] in the unmodified β2 and β3 chains of globin. Selected reaction monitoring LC/MS/MS was used to detect the presence of *S*-hexahydro-1*H*-azepine-1-carbonyl cysteine (HHAC-Cys) in the globin hydrolysates isolated from the molinate-treated rats and to confirm the structure of the modification. These experiments demonstrate the ability of molinate to covalently modify proteins *in vivo* in a dose dependent manner *via* carbamylation at Cys-125, similar to the modification produced by disulfiram and *N, N*-diethyldithiocarbamate. The ability of molinate to covalently modify cysteine residues provides a potential mechanism to account for enzyme inhibition following molinate exposure and suggests that enzymes with cysteine residues in their active site may be inhibited by molinate. These results may also extend to other structually related pesticides.

1555 GLUTATHIONE DEPLETION IN RAT PRIMARY HEPATOCYTES AND LIVER BY N-METHYLDITHIOCARBAMATE (NMDC) AND N, N-DIMETHYLDTHIOCARBAMATE (DMDC).

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Several decomposition and metabolic pathways are shared by the commercially used monoalkyl and dialkyl dithiocarbamates that can alter intracellular thiol status. This investigation examines the role of glutathione in the cytotoxicity of dithiocarbamates and investigates the primary mechanisms responsible for glutathione depletion by NMDC and DMDC in cultured primary hepatocytes and perfused rat liver. Because monoalkyl dithiocarbamates can acylate intracellular thiols *via* generation of an isothiocyanate (ITC) intermediate, the contribution of ITC to the cytotoxicity of NMDC was also determined. Viability was assessed in hepatocyte cultures by LDH release while reduced, oxidized and S-methylthiocarbamoyl glutathione (GSMITC) levels were determined using HPLC and LC/MS/MS in both cultured hepatocytes and bile samples. The cytotoxicity for both NMDC and DMDC was inversely correlated with intracellular levels of GSH. Although hepatocytes exposed to either NMDC or DMDC had diminished intracellular levels of GSH, DMDC appeared to oxidize GSH to GSSG whereas NMDC exposure resulted in generation of GSMITC. In bile samples, GSMITC was also detected

within 5 minutes of infusion of NMDC into the liver, however GSSG was not elevated upon DMDC exposure. These results demonstrate that distinct processes involved in lowering glutathione levels in hepatocytes following incubation with NMDC or DMDC. For the monoalkyl dithiocarbamate generation of the reactive ITC followed by acylation of cellular thiols appears to play a significant role. The rapid appearance of GSMITC in bile samples from NMDC infused livers indicate that enzymatic systems may be involved. In contrast, dialkyl dithiocarbamates altered intracellular thiol status in cultured hepatocytes by oxidation of GSH but a similar observation was not obtained in the perfused liver experiments. It is anticipated that the results obtained for these two dithiocarbamates may be applicable to other monoalkyl and dialkyl dithiocarbamates.

1556 ETHYLENE-*BIS*-DITHIOCARBAMATES CAN INHIBIT ISOLATED BRAIN AND LIVER MITOCHONDRIA.

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Dithiocarbamate (DTC) pesticides, such as maneb (manganese ethylene-*bis*-dithiocarbamate (EBDTC)), have been reported in the literature to cause parkinsonian symptoms in certain individuals. Recent data from other labs have also demonstrated that mice treated with maneb show neurodegeneration, which is specific to the striatum. Our hypothesis is that maneb can initiate this neurodegeneration through the production of reactive oxygen species. One mechanism by which maneb could facilitate the formation of free radicals is through inhibition of mitochondrial respiration. Brain and liver mitochondria, isolated from C57 Bl/6 male mice, were treated with various concentrations of DTC compounds in oxygen consumption chambers equipped with oxygen sensing electrodes. Two classes of DTCs, EBDTC and diethyldithiocarbamate (DEDC), were used in order to determine the relative importance of the organic or metal constituent in inhibiting respiration. Maneb, zineb (Zn(EBDTC)), (NH_4)EBDTC, and $Mn(DEDC)_2$ were all shown to inhibit mitochondria in the nmol / mg protein range when glutamate / malate was used as a substrate for complex I. Furthermore, brain mitochondria were more severely inhibited than liver mitochondria, suggesting a mechanism for brain selectivity. The potency of compounds for brain mitochondria was $Mn(DEDC)_2$ > EBDTC > maneb ≈ zineb. For liver mitochondria the potency was zineb > $Mn(DEDC)_2$ > EBDTC > maneb. This data suggests that, for mitochondrial inhibition, the organic moiety plays a more significant role than the metal in reducing mitochondrial respiration.

1557 MANEB AND PARAQUAT EFFECTS ON ANTIOXIDANT SYSTEMS IN PC12 CELLS.

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Environmental chemical exposures are potential risk factors for Parkinson's disease (PD). Recently, combined paraquat (PQ) and maneb (MB) exposure were shown to produce synergistic effects in the nigrostriatal dopamine system *in vivo*. Although PQ is a redox cycling compound, the mechanisms by which MB or combined agrichemical exposures produce neurotoxicity are unresolved. In this study, PC12 cells were used as a model to assess the effects of MB and evaluate mechanisms of neurotoxicity following combined agrichemical exposure. Cultures were exposed to various concentrations of MB, PQ and combined PQ+MB for 6-48 h then assayed for cell viability by measuring lactate dehydrogenase (LDH) release into the culture media. LDH levels did not differ between control and treated cells until 48 hours, where there was a slight concentration-dependent elevation in both the MB and combined treatment groups. To assess intracellular redox status, total glutathione (GSH) was enzymatically measured following treatments. Total intracellular GSH increased between 6h and 24h following MB treatment, with maximal levels reaching 225% of control. By 48h, GSH levels remained elevated in cells treated with the highest concentration of MB alone. In combined MB+PQ treatment groups, GSH levels declined by 30% in the lower dose combination groups after 24 hours, compared to MB treatment alone. Higher dose combinations resulted in a more robust elevation of intracellular GSH, which increased with treatment duration. To further evaluate antioxidant defense systems, CuZn- and Mn-SOD protein levels were monitored by immunoblot analysis. At 6 hours, CuZn SOD levels were elevated following treatment with both low and high dose combinations compared to MB alone. SOD expression was increased after 24h by MB and PQ treatments alone. However, SOD levels declined below control in both dose combination groups. These findings suggest that interactions between MB and PQ disrupt cellular antioxidant defense systems, ultimately resulting in altered cellular homeostasis and function. Supported by NIH ES10791 and ES01247.

1558 GENDER DIFFERENCES ASSOCIATED WITH DEVELOPMENTAL AND ADULT EXPOSURE TO PARAQUAT AND MANEB.

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Parkinsons disease (PD), an aging neurological disorder, for unknown reasons is more prevalent in males than females. While the etiology of PD remains unclear, environmental factors are strongly implicated. We have previously demonstrated that combined exposure to paraquat (PQ) and maneb (MB) produces an early irreversible PD phenotype, including potentiated reductions in locomotor activity and loss of nigrostriatal dopamine neurons in adult male mice. To determine whether gender effects are observed with this model, male and female C57BL/6 mice were exposed developmentally to saline, 0.3 mg/kg PQ, 1 mg/kg MB or PQ+MB from postnatal (PN) day 5-19. A subset of these mice was re-challenged as adults (AD) with saline, 10 mg/kg PQ, 30 mg/kg MB or PQ+MB. A separate group was exposed only as adults. PN exposure to PQ+MB decreased locomotor activity similarly in males and females at 6 weeks of age. By 6 months of age, PQ+MB treated males showed a progressive decrease in activity compared to 6 weeks of age, whereas the females showed a progressive recovery, with activity levels returning to control levels at this time-point. Consistent with this, PN only exposure significantly decreased striatal DA, DOPAC and HVA levels in males and females exposed to PQ alone and PQ+MB, with greater decreases observed in males. PN + AD exposure significantly decreased striatal DA, DOPAC and HVA in all male treatment groups to a greater extent compared to PN alone exposure. In contrast, DA and metabolite levels in PN + AD females treated with PQ and MB alone and in combination were not significantly reduced. The males showed a significantly greater decrease compared to the females. Further, females exhibited full recovery, but males only partial recovery of locomotor activity following AD only exposure to PQ+MB. These data suggest protective mechanism(s) in females that may be provided by estrogen, which has repeatedly been demonstrated to be a neuroprotective agent against dopaminergic neurotoxicants.

1559 FIXED RATIO (FR) SCHEDULE-CONTROLLED BEHAVIOR IN RATS FOLLOWING EXPOSURES TO PARAQUAT AND MANEB.

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Previous studies have shown that combined exposure to paraquat (PQ) and maneb (MB) produce potentiated, selective and irreversible neurotoxicity to the nigrostriatal dopamine (DA) system in C57BL/6 mice. This includes sustained reductions in locomotor activity, loss of DA and of DA neurons in the substantia nigra and serves as a model of early environmental Parkinsonism (PD). To determine the species generality of these effects, and attempt to develop a comparable rat model of PD, male Long Evans rats were trained to lever press for food reinforcement on a FR schedule. Reductions in response rates on FR schedules have been related to DA loss in the nigrostriatal DA system as induced by the neurotoxicant 6-OHDA. Thus, it was utilized here as a means of preclinical detection of DA loss. The FR requirement was increased to an FR 40 and continued until a stable baseline was attained. Rats were then treated twice a week for 3 weeks with saline, 10mg/kg PQ, 30mg/kg MB, or PQ+MB for a total of 5 doses. PQ and MB administered alone produced marginal decreases in overall response rates (OR) and run rates (RR) compared to control on the FR schedule. Administration of combined PQ+MB resulted in decreases in both measures that appeared to increase in magnitude with successive doses. For 24 hr post-dosing, OR and RR in the PQ or MB alone groups had fully recovered to control levels, whereas rates in the combined PQ+MB showed residual reductions and only returned to control levels 48 hr post-dosing. This 24 hr post-dosing reduction in FR rates in the PQ+MB group also seemed to increase with successive treatments. Rats are known to be resistant to MPTP, the commonly used neurotoxicant model of PD. Unlike MPTP, however, PQ+MB seem to exert greater effects in the rat than were observed in mice where total regimens of 12 injections using these same doses of PQ+MB were administered without any signs of overt toxicity. Supported by ES10791, ES01247, & ES007026

1560 PARAQUAT TOXICITY IS NOT MEDIATED BY THE DOPAMINE TRANSPORTER.

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Parkinson's disease is characterized by the loss of nigrostriatal dopaminergic neurons and the resultant loss of dopamine input to the striatum is thought to cause the clinical symptoms. Pesticide exposure is considered to be a risk factor for

Parkinson's disease. The herbicide paraquat has been suggested as a candidate environmental agricultural chemical risk factor due to its structural similarity to 1-methyl-4-phenylpridinium ion (MPP+), the active component of dopaminergic neurontoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP). In addition, mice treated with combined paraquat and maneb exhibit the toxicity in the nigrostiatal dopamine system. Our Lab. had generated an inducible system for dopamine transporter (DAT) in SK-N-MC neuroblastoma cell line. Using this system, we have already shown that MPP+ toxicity corresponded with DAT expression. To determine the role of DAT in paraquat neuron toxicity, we tested the effect of paraquat in the DAT-mediated dopamine uptake. Only the 10mM paraquat partially inhibited the dopamine uptake (~20%). Furthermore we treated the inducible DAT cell line with serial concentrations of paraquat (0, 0.05, 0.1, 0.2, 0.25, 0.5, 0.75, 1 mM). The toxicity corresponded with the concentration of paraquat (the cell viability was from 90%-26%), but was independent of the DAT expression level while the MPP+ toxicity corresponded to the DAT expression level. These findings indicate that while paraquat and MPP+ have similar chemical structures, paraquat is not transported by DAT. Future studies should be aimed at identifying the mechanism by which paraquat targets the dopamine neuron. (Supported by NIH ES-09248, ES-07784, and NS-37031)

1561 MODULATION OF MURINE STRIATAL DOPAMINE TRANSPORTER EXPRESSION BY THE PYRETHROID INSECTICIDE PERMETHRIN.

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The pyrethroid insecticide permethrin may play a role in the development of Parkinson's disease as a consequence of Gulf War Syndrome. Previous work in our laboratory has shown that an i.p. dose of 1.5 mg/kg permethrin in the C57BL/6 mouse causes a significant increase in striatal dopamine uptake to 125% of control values. Recently, we have dosed C57 mice with either 0.8 or 1.5 mg/kg permethrin (3 i.p. injections over 2 weeks). To assess reversibility of effects on striatal dopamine transporter (DAT), a portion of the mice were sacrificed on the day following the last dose (t=0), while a second group was sacrificed 2 weeks post last treatment (t=2), and a third group 4 weeks post last treatment (t=4). At t=0, DAT protein as assayed by Western blotting was increased 119% and 107% in the 0.8 and 1.5 mg/kg group, respectively, over that of control mice. DAT binding was analyzed by incubating the ligand [^{3}H]GBR 12935 with striatal synaptosomes from control and treated mice. [^{3}H]GBR 12935 binding was increased 156% and 118% in mice treated with 0.8 and 1.5 mg/kg permethrin, respectively. Preliminary evidence suggests that this effect is persistent, since DAT protein was likewise elevated in the t=2 group of mice. Due to its effects on mouse DAT, the ability of permethrin to synergize the effects of the neurotoxin MPTP was ascertained. MPTP (30 mg/kg) was administered to C57BL/6 mice on day 1 of the treatments, followed the next day by administration of various doses of permethrin from 0.8 mg/kg to 200 mg/kg. Striatal dopamine and its metabolite, DOPAC, were determined by HPLC-ECD. Only the highest dose of permethrin (200 mg/kg) in combination with MPTP was able to deplete dopamine significantly greater than MPTP alone. However, DOPAC titers were significantly elevated at doses above 50 mg/kg permethrin, suggesting an increase in dopamine turnover. Taken together, these data suggest that insecticides such as permethrin may have subtle effects on the dopaminergic system, and may be involved in the etiology of Parkinson's disease.

1562 DECREASE OF DOPAMINE LEVELS AFTER PYRETHROID TREATMENT.

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Cyfluthrin and Cyhalothrin, Type II pyretroid insecticides, are used topically for the control of ectoparasites. Cyfluthrin and Cyhalothrin when injected peripherally to rat produced a severe syndrome characterized by tremor, salivation and choreoathetosis. Because of toxicological data are needed to further define the use safety of these insecticides, the objective of the present study was to investigate neurochemical effects following the administration of Cyfluthrin (14 mg/kg/day, orally for 6 days) or Cyhalothrin (8 mg/kg/day, orally, for 6 days) in male Wistar rats (n =6/group). Animals were sacrificed 24 hours following pyrethroid administration and their brains were rapidly removed. The frontal cortex, 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 effect was produced by these pyrethroids. Cyfluthrin decreased DA, DOPAC and HVA levels in frontal cortex (29%, P<0.01; 48%, P<0.001and 20%, P<0.05), midbrain (22%, P<0.05; 23%, P<0.01and 33%, P<0.01) and striatum (23%, P<0.05; 21%, P<0.05 and 29%, P<0.001) respect to corn oil controls. Similarly, Cyhalothrin decreased DA, DOPAC and HVA levels in frontal cortex (64%, P<0.01; 30%, P<0.01and 14%), midbrain (19%, P<0.05; 31%, P<0.01and 13%) and striatum (28%, P<0.05; 3% and 39%, P<0.001) respect to corn oil controls. These findings indicate that a lower activity of dopaminergic system exists in the action of Cyfluthrin and Cyhalothrin. This work has been supported by projects No. PB9701236, (DIGICYT), No. 08.8/0002/98 (CAM) & No. 99/0936 (FIS), Spain.

1563 NEURON DEGERNERATION INDUCED BY SUBCHRONIC APPLICATION OF N, N-DIETHYL M-TOLUAMIDE (DEET) AND PERMETHRIN, ALONE AND IN COMBINATION TO RATS.

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Histopathological alterations in the brain of adult male rats were evaluated following a daily dermal dose of DEET (40 mg/kg in 70% ethanol) or permethrin (0.13 mg/kg in 70% ethanol) or a combination of the two for 60 days. Control rats received a daily dermal dose of 70% ethanol for 60 days. Animals were perfused and brains were processed for morphological and histopathological analyses following the above regimen. In animals receiving either DEET or Permethrin, degenerating (eosinophilic) neurons were diffusely observed in distinct regions of the motor and somatosensory cortex, the hippocampus, and the cerebellum. In contrast, dying neurons were infrequent in animals receiving both DEET and Permethrin. The density of surviving neurons in cerebral cortex, hippocampus and cerebellar Purkinje cell layer of these animals, however, was dramatically less than control animals, suggesting that in animals receiving both DEET and permethrin neuronal cell death occurred earlier than animals receiving either DEET or permethrin alone. Analysis of glial fibrillary acidic protein immunoreactivity revealed significant hypertrophy of astrocytes in all three treated groups with maximal changes in rats receiving both DEET and permethrin. Further, surviving neurons in the latter group exhibited abnormal dendrites. Thus, sub-chronic dermal application of DEET and permethrin either alone or in combination leads to diffuse neuronal cell death in cerebral cortex, hippocampus and cerebellum of the adult brain; the neurotoxic effect is more pronounced when DEET and permethrin are applied together. Collectively, the above alterations can lead to many physiological, pharmacological and behavioral abnormalities, particularly motor and sensory deficits and learning and memory dysfunction. Supported in part by the US Army Medical Research and Materiel Command under Contract DAMD 17-99-1-9020.

1564 MECHANISMS OF PYRETHROID NEUROTOXICITY: IMPLICATIONS FOR CUMULATIVE RISK ASSESSMENT.

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The Food Quality Protection Act (FQPA) of 1996 requires the USEPA to consider the cumulative effects of exposure to pesticides having a "common mechanism of toxicity." This presentation reviews published and proprietary data on the neurotoxicity and mechanisms of toxic action of pyrethroid insecticides in mammals as pertains to this statute. The principal effects of pyrethroids in mammals are various signs of excitatory neurotoxicity. Historically, pyrethroids were grouped into two subclasses (Type I and Type II), based on chemical structure and the associated syndrome of intoxication (T (tremor) or CS (choreoathetosis with salivation). However, this classification system is inadequate for identification of common toxic effects. In particular, it does not reflect the diversity of toxic signs evident following oral administration of the various pyrethroids. With respect to mechanisms of toxicity in mammals, pyrethroids act *in vitro* on a variety of putative biochemical and physiological target sites that merit consideration as sites of toxic action. Voltage-sensitive sodium channels, the sites of insecticidal action, are also important target sites in mammals. However, unlike insects, mammals have many sodium channel isoforms that vary in their biophysical and pharmacological properties, including their sensitivity to various pyrethroids. Pyrethroids also act on some isoforms of voltage-sensitive calcium and chloride channels and these may contribute to toxicity. Effects on peripheral-type benzodiazepine receptors are unlikely to be a principal cause of pyrethroid intoxication but may contribute to convulsions caused by actions at other target sites. Other putative target sites that have been identified *in vitro* do not appear to play a major role in pyrethroid intoxication. The diverse toxic and pharmacologic effects of pyrethroids suggest that simple additivity models, based on combined actions at a single target, are not appropriate to assess cumulative risk.

1565 AN HPLC METHOD FOR THE DETERMINATION OF MAJOR PERMETHRIN METABOLITES AND PERMETHRIN METABOLISM IN HUMAN LIVER.

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An efficient high performance liquid chromatography (HPLC) method was developed to separate and quantitate two permethrin isomers and three major metabolic products (phenoxybenzyl alcohol, phenoxybenzoic acid and phenoxybenzaldehyde), which were produced in studies of *in vitro* human metabolism using human liver fractions. Two hundred and thirty nanometers was found to be the most sensitive detection wavelength for permethrin isomers and metabolites among 4 previously used wavelengths (210, 230, 254 and 270 nm). Detection limits at 230 nm were 12 nM for phenoxybenzoic acid and 24 nM for all other compounds. A gradient elution using two solvents was used with the initial mobile phase condition of 50 % of solvent B (100 % water adjusted to pH 1.7 with phosphoric acid) and 50 % of solvent A (90 % acetonitrile and 10 % water, v/v). A pH of less than 2 was required for solvent B to fully protonate and separate an ionizable metabolite, phenoxybenzoic acid. Retention times were 3.7, 4.1, 5.9, 10.2 and 10.5 minutes for phenoxybenzyl alcohol, phenoxybenzoic acid, phenozybenzaldehyde, trans-permethrin and cis-permethrin, respectively. When trans-permethrin was incubated with human liver fractions, phenoxybenzyl alcohol and phenoxybenzoic acid were the major products. Trans-permethrin incubation with 12 individual human cytochrome P450 isozymes did not produce any significant amounts of metabolites. Addition of NAD^+ to human liver microsomal fraction significantly increased the production of phenoxybenzoic acid with a minimum amount of phenoxybenzyl alcohol detected. An incubation of phenoxybenzyl alcohol and phenoxybenzaldehyde metabolism with horse liver alcohol dehydrogenase and yeast aldehyde dehydrogenase resulted in significant levels of phenoxybenzaldehyde and phenoxybenzoic acid formation respectively. These results suggested that permethrin hydrolysis products in human liver may be further metabolized by NAD^+ requiring alcohol and aldehyde dehydrogenases without cytochrome P450 involvement.

1566 INDUCTION OF CYTOCHROME P4501A1/2 AND P4504A1 ACTIVITIES BY PYRETHROIDS.

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The objective of the present study was to investigate the effects of repeated exposure to Cyfluthrin or Cyhalothrin, Type II pyretroid insecticides, on hepatic mixed function oxidase activities in male Wistar rats following oral administration. Animals were treated with Cyfluthrin (20 mg/kg/day orally for 6 days) or cyhalothrin (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 and microsomal pellets were prepared and stored at –80°C for enzyme determinations. O-demethylation of metoxyresorufin (MROD) and O-deethylation of ethoxyresorufin (EROD) were determined from appearance of resorufin measured fluorimetrically. Finally 11- and 12-hydroxylations of lauric acid were also determined on the microsomal fraction by a HPLC method with radiochemical detection. **Effects of pyrethroids on rat hepatic mixed function oxidase system:** Results are significantly different from the control value at *P<0.05, **P<0.01, or ***P<0.001 These findings may prove a valuable contribution for risk assessment of these insecticides. This work has been supported by projects No. PB9701236, (DIGICYT), No. 08.8/0002/98 (CAM) & No. 99/0936 (FIS), Spain.

	Cyfluthrin	Cyhalothrin
EROD (CYP1A1) (percentage change over control)	85%**	195%***
MROD (CYP1A2) (percentage change over control)	39%**	59%**
LAURIC ACID 12-HYDROXYLASE (CYP4A1) (percentage change over control)	135%**	66%**
LAURIC ACID 11-HYDROXYLASE (percentage change over control)	168%**	77%*

1567 COMPARISON OF DIETARY AND ORAL GAVAGE ADMINISTRATION OF BIFENTHRIN IN DEVELOPMENTAL TOXICITY STUDIES IN SPRAGUE-DAWLEY RATS.

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Bifenthrin, a synthetic pyrethroid insecticide/miticide, was tested in two developmental toxicity studies in Sprague-Dawley rats. Bifenthrin was administered at 5 ml/kg/day *via* gavage to groups of 25 rats as a solution in corn oil at dosages of 0, 0.5, 1.0 and 2.0 mg/kg/day on days 6 through 15 of gestation. Intermittent tremors were seen among animals receiving 2.0 mg/kg between days 10 and 19 of the study. There were no treatment-related effects observed on body weight gains, food consumption, pregnancy, number of implantations, resorptions, litter size, external or internal anomalies (c-sections on day 20). There were no effects at 1.0 mg/kg or below. In the second study, bifenthrin was administered in the diet at dose levels of 0, 30, 90 and 200 ppm on days 6-20 of gestation. Treatment-related clinical signs included tremors, hypersensitivity to sound, piloerection and splayed hindlimbs at 200 ppm. Significant reductions in maternal body weights and gains, adjusted maternal body weights and food consumption were noted at 200 ppm. At c-section on day 20, there were no significant differences in number of implants, pre-implantation loss, litter-size, the percentage of live and dead fetuses, percentage of early and late resorptions, fetal body weights and fetal sex ratios or fetal or litter incidence of external, visceral or skeletal findings. The No Observed Effect Level was 90 ppm (7.4 mg/kg/day), based on clinical signs at 200 ppm (16.3 mg/kg/day). The use of corn oil as a vehicle allows for dosing using oral gavage and measurement of the dose each animal receives, however, it results in delivery of a bolus dose into the stomach in an unrealistic vehicle. Administration of bifenthrin in the diet more closely approximates the route of exposure for the human population exposed to small amounts of bifenthrin in their diet.

1568 PESTICIDE TOXICITY IN THYMOCYTES IS RELATED TO OXIDATIVE STRESS.

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Pesticides are widely used in the environment, and exposure to multiple pesticides is very likely, either concurrently or sequentially, over one's lifetime. We have found that selected pesticides lindane (L; an organochlorine), malathion (M; an organophosphate), and permethrin (P; a pyrethroid) are cytotoxic to murine (C57Bl/6) thymocytes in both concentration- and time- dependent manner, *in vitro*. Based on minimum cytotoxicity ($\leq LC_{30}$ with individual pesticides), the following concentrations were chosen: 50 µM L, 75 µM M, 150 µM P, 50 µM L + 75 µM M mixture, and 50 µM L + 150 µM P mixture. These concentrations of pesticide caused 16.6, 21.5, 29.0, 59.8 and 53.3 % cell death (examined by 7 aminoactinomycin-D assay), respectively. To investigate the role of oxidative stress in causing cytotoxicity, we monitored both the pro- and anti-oxidant status of cells. The generation of hydrogen peroxide (H_2O_2), an indicator of pro-oxidant status of cells, was monitored by CM-H_2 DCFDA assay. Exposure to above concentrations of pesticides for 15 min augmented the H_2O_2 production in cells (L 37.2, M 39.2, P 40.5, L+M mix 52.4 and L+P mix 50.6 % over control). The effects of above concentrations of pesticide exposure to the cells for 12 hr on activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-Rx) enzymes were studied to evaluate the antioxidant status of cells. The activity of CAT was not significantly altered by these treatments. SOD levels were not altered by individual pesticide exposure, but mixtures of pesticides caused increase in SOD levels (Units/mg; L 29.1, M 29.4, P 28.0, L+M mix 37.5, L+P mix 41.1). GSH-Px and GSH-Rx levels were not affected with individual pesticide exposures, but significantly reduced with pesticides mixtures (GSH-Px Units/mg: 30.9, 28.7, 32.8, 23.2, 21.8; GSH-Rx Units/mg: L 33.9, M 36.1, P 36.6, LM mix 25.7, LP mix 26.1). These results indicate that lindane, malathion and permethrin exposure cause immunotoxicity, at least in part, *via* oxidative stress.

1569 TOXICOLOGICAL ASSESSMENT OF THE OXANILIC ACID METABOLITE OF ALACHLOR.

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Alachlor is a selective herbicide used on corn and other crops. The *tert*-amide oxanilic acid metabolite of alachlor (alachlor OXA) is an environmental degradate that is formed in soil by microbial action and is occasionally detected in ground and surface water. Thus, a number of toxicological studies were conducted with alachlor OXA in order to evaluate the potential human or ecological effects that may be associated with this metabolite. These included acute and subchronic studies in rats, a battery of *in vitro* and *in vivo* genotoxicity studies, and acute studies in fish, aquatic invertebrates, earthworms and algae. These studies demonstrated that alachlor OXA is relatively non-toxic to all species tested. No mortality occurred in rats following a single oral dose of 5000 mg/kg. The No-Observed-Effect-Levels (NOELs) in 28-day and 90-day rat feeding studies were 10, 000 ppm (approximately 792 mg/kg/day) and 13, 000 ppm (approximately 922 mg/kg/day)(highest level tested), respectively. No evidence of genotoxicity was observed in Ames, mouse lymphoma, *in vitro* chromosomal aberration or mouse micronucleus assays. Alachlor OXA was also practically non-toxic in acute studies with several aquatic and terrestrial organisms. No mortality was observed in rainbow trout (*Oncorhynchus mykiss*), *Daphnia magna* or earthworms (*Eisenia foetida*) at the high-

est concentrations tested (100, 95 and 1000 ppm, respectively). The EC_{50} values in *Selenastrum capricornutum* and *Navicula pelliculosa* were greater than 100 ppm. Thus, these results demonstrate that the acute and subchronic toxicity of alachlor OXA is substantially less than that of parent alachlor, and indicate that the very low levels of alachlor OXA which are sometimes found in ground and surface water will not pose a significant risk to human health or the environment.

1570 HEPATIC RESPONSES TO CHLOROACETANILIDE HERBICIDE α-CHLORINE DISPLACEMENT DISTINGUISHED BY INDUCTION WITH BOTH ALACHLOR AND METOLACHLOR.

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Previous studies indicate that acute hepatotoxicity of alachlor [2-chloro-2', 6'-diethyl-*N*-(methoxymethyl)-acetanilide; > 400mg = 1.85 mmol/kg] to male Long-Evans rats is associated with sustained depletion of reduced glutathione (GSH). However, comparable GSH loss occurs with metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide; ≦ 2.6 mmol/kg] in the absence of acute hepatotoxicity (Dalton et al., *Toxicol. Sciences.* 60:54, 2001). Unlike alachlor, metolachlor is not metabolized to quinoneimine, but both chloroacetanilides contain an acetyl α-chlorine group. In the present studies, neither alachlor nor metolachlor induced oxidative damage, assessed as thiobarbituric acid-reactive malondialdehyde of liver samples (prepared with antioxidant BHT). Glutathione-*S*-transferase activity (GST), with chlorodinitrobenzene as substrate, showed statistically significant dose-dependent increases 24 hr after exposure to both alachlor and metolachlor, which immunoblot analysis indicated was not due to an increase in oxidant-induced isoform α or μ. Collectively, these data suggest that alachlor-induced GSH loss results from conjugation of the α-chlorine group by nucleophilic substitution and is insufficient to cause oxidant-mediated hepatotoxicity. Additional comparative studies using enzymatic and immunoblot analyses of cytochrome P450s indicated that alachlor and metolachlor (0.5 mmol/kg, 4 daily doses) induced CYP2B1/2 to a similar extent, but neither induced CYP1A1, 1A2 or 3A1. In conclusion, sustained GSH depletion and induction of GST and CYP2B1/2 by metolachlor, but in the absence of hepatotoxicity, indicate that these responses are related to adaptation to chloroacetanilide exposure. Further, they suggest that alachlor hepatotoxicity can be attributed to a structural group other than the acetyl α-chlorine. (support: NIH ES07046 & University. La-Monroe Coll. Pharmacy)

1571 EFFECTS OF CARBARYL ON THE MOTOR ACTIVITY OF SPONTANEOUSLY HYPERTENSIVE (SHR) AND NORMOTENSIVE (WKY) RATS.

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SHR rats have been widely used to investigate the etiology and mechanisms of hypertension. Recent evidence suggests SHR rats have an increased sensitivity to cholinesterase inhibitors. In an effort to develop animal models of susceptibility for use in risk assessment, this experiment compared the effects of the carbamate pesticide carbaryl on the motor activity of SHR and WKY rats. Adult male rats of each strain (N=18) were tested daily during 30-min sessions in a photocell device that separately recorded horizontal and vertical activity. Once activity levels stabilized, carbaryl (5-100 mg/kg) was administered p.o. in corn oil, once weekly, 60-min presession to all rats. Baseline motor activity was consistently higher in SHR than in WKY rats; SHR rats also showed no habituation of activity across initial test sessions, in contrast to the WKY rats. Carbaryl produced dose-related decreases in horizontal and vertical activity in SHR rats. Over the same dose range, carbaryl decreased vertical activity but had no effect on the horizontal activity of WKY rats. Determination of cholin-esterase activity in blood showed that activity was higher in SHR than WKY rats after vehicle, but carbaryl (15 mg/kg) produced a comparable degree of inhibition in both strains. Our behavioral results indicate that SHR rats are sensitive to carbaryl and WKY rats are remarkably insensitive to this pesticide. This abstract does not necessarily reflect USEPA policy.

1572 EXPOSURE TO THE TRIAZOLE FUNGICIDE TRIADIMEFON CAN PRODUCE BOTH INDUCTION AND EXPRESSION OF BEHAVIORAL SENSITIZATION.

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Triadimefon (TDF), a triazole fungicide widely used on fruit and grain crops, increases both locomotor and stereotypical activity in rodents. TDF, like cocaine, blocks reuptake of dopamine (DA), leading to increased extracellular DA levels in the CNS. A recent study in this laboratory, in which mice were administered 24 doses of 50 mg/kg TDF (TDF50) over 12 weeks, found a progressive increase in vertical and ambulatory activity suggesting its ability to result in behavioral sensitization, a process thought to be associated with relapse to drug abuse and addiction. The current study hypothesized that TDF itself, as a psychostimulant, could both induce behavioral sensitization, i.e., the progressive increase in the locomotor response to psychostimulants, as well as lead to the expression of sensitization, the heightened locomotor activity response following re-challenge after withdrawal. Towards this end, C57BL/6 mice were dosed with 50 (TDF50) or 75 mg/kg TDF (TDF75) or with saline ip, twice a week for a total of 14 doses and locomotor activity measured. Re-challenge doses of saline and of TDF were given after a 2 week and 2 month withdrawal period. Vertical activity showed the greatest sensitivity to TDF and was increased across the dosing period. At the 2 week re-challenge, TDF75, but not TDF50, further increased vertical activity to levels higher than those exhibited during dosing. The 2 month TDF re-challenge did not reveal sensitization, possibly due to progressive neurotoxicity which may dampen neurochemical sensitization. This study reveals for the first time that TDF exposure can produce both the induction and expression of behavioral sensitization, a phenomenon commonly associated with dopaminergic and glutamatergic neurochemical changes. TDF exposure could therefore serve as a risk factor for disturbances in behavioral functions mediated by these neurochemical systems. Supported by ES10791, ES01247, and ES007026

1574 COMPARATIVE *IN VITRO* AND EX-VIVO STUDIES ON PROPANIL MYELOTOXICITY.

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3', 4'-Dichloropropionanilide (propanil) has been shown to possess methemoglobinemic activity in both animals and humans possibly through its metabolites 3, 4-dichloroaniline (DCA) and N-hydroxy 3, 4-DCA. Propanil also exerts immunotoxic and myelotoxic effects in experimental models. The aims of this study were (i) to compare *in vitro* the myelotoxic effects of propanil and its metabolite DCA on mouse bone marrow (BM) precursors by means of colony forming unit (CFU) assays of the myeloid - granulocyte and macrophage - lineage (CFU-GM), the erythroid lineage (CFU-E) and the less mature burst-forming unit erythroid (BFU-E); (ii) to compare the *in vitro* data with those obtained in ex-vivo experiments performed on BM cells isolated from mice exposed acutely to propanil *in vivo*. In the vitro assays, propanil and DCA concentration-dependently reduced the number of erythroid colonies. Propanil IC50s were 69 ± 12 and 18 ± 11 microM in the CFU-E and BFU-E assays, respectively, while those of DCA were 26 ± 7 and 13 ± 5 microM, respectively. Propanil and DCA were equally potent inhibitors of CFU-GM colony formation *in vitro* (IC50 = 170 microM). *in vivo* exposure to a single dose of propanil (0, 50, 100 and 200 mg/kg i.p.) caused a dose-dependent decrease of CFU-E, BFU-E and CFU-GM progenitors 7 days post-exposure. Compared to controls, 100 and 200 mg/kg propanil reduced CFU-E colony formation by approximately 40 and 50%, respectively, and BFU-E by 24 and 38%, respectively. CFU-GM colony formation was also inhibited to a similar degree. Peripheral blood parameters were not affected by any dose of propanil 1 week after injection. In summary, propanil elicits myelotoxic effects both *in vitro* and *in vivo*. The higher potency of DCA toward the BFU-E cells compared to that of propanil suggests a role for propanil metabolites in its toxicity.

1575 CLODINAFOP IS A PEROXISOME PROLIFERATOR IN THE MOUSE LIVER.

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Clodinafop-propargyl (CLF) induced hepatocellular neoplasms at high doses in male mice. In the absence of any mutagenic activity, the induction of liver tumors was considered to be due to a non-genotoxic mechanism. Increased liver weight and hepatocellular hypertrophy was observed in subchronic studies, suggesting an induction of liver enzymes and a mitogenic response. Two studies with male mice were initiated comprising biochemical liver parameters (cytochrome P450 monooxygenases and peroxisomal fatty acid oxidation) and bromodeoxyuridine-based cell proliferation analysis (treatment periods up to 28 days). Major treatment-related biochemical alterations comprised an induction of cytochrome P450 isoenzymes of gene subfamily Cyp4a as indicated by a strong increase in immunochemically detectable Cyp4a proteins and by associated lauric acid 12-hydroxylase activity. Peroxisomal fatty acid oxidation activity was markedly increased. Cell proliferation analysis revealed a strong but transient stimulation of hepatocellular proliferation, accompanied by hepatocellular hypertrophy. In addition, morphometry

of hepatocellular peroxisomes revealed an increase in number and percent area of these organelles. These data identify CLF as a strong peroxisome proliferator with mitogenic and enzyme inducing activity in the mouse liver. Therefore, the carcinogenic response observed with CLF in the mouse liver is inappropriate for extrapolation to humans.

1576 ASSESSMENT OF THE POTENTIAL INTERACTION OF ^{14}C-THIAMETHOXAM WITH HUMAN P-GLYCOPROTEIN.

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P-glycoprotein (PGP, encoded by mdr1) is a member of the ABC transporter superfamily and is expressed in endothelial cells of the capillary bed in the brain, intestine, liver, kidney and other tissues. PGP serves as a barrier to keep toxins out of these tissues by actively pumping chemicals that bind to PGP across the cell membrane and out of the cytoplasm. Numerous insecticides have been demonstrated to interact with human PGP [Bain et al., 1997, *EHP*, 105: 812-818]. In order to evaluate the potential of the new insecticide, thiamethoxam to interact with PGP, two experiments were performed in control and human PGP-expressing LLC-PK1 cells derived from the human kidney. The first, a time-dependence experiment with ^{14}C-thiamethoxam, was performed to determine the apparent permeability(P_{app}) and bidirectional transport of thiamethoxam in the control and polarized cell monolayers. The P_{app}(7.4 - 8.5 x 10^{-6} cm/sec for a time cource of 15 to 120 minutes) in the control cell (clone A) monolayers is consistent with a membrane-permeable substance. The polarization ratio ($P_{app\ B\ to\ A}/P_{app\ A\ to\ B}$) in the human PGP-expressing LLC-PK1 cells (clone 15B-J) was essentially equal to that in the control cells, at each timepoint measured. In contrast, the 15B-J polarization ratio with digoxin (the positive control for PGP transport) was higher than the control cell ratio, demonstrating that digoxin was transported. The mass balance analysis at 120 minutes resulted with a % recovery of 98±1. The results demonstrated that thiamethoxam was not transported by PGP. The second experiment (PGP-inhibition) determined that the PGP-facilitated transport of digoxin was not inhibited by co-incubated thiamethoxam, as was evident by the consistent digoxin polarization ratios measured over the thiamethoxam concentration range of 0 to 100 µM whereas, ketoconazole inhibited the digoxin transport. The results of the study clearly demonstrated that thiamethoxam is not a substrate or inhibitor of human PGP-mediated efflux.

1577 BLOCK OF COCKROACH NEURON SODIUM CHANNELS BY INDOXACARB INSECTICIDE.

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Indoxacarb is a newly developed insecticide with high insecticidal activity and low toxicity to nontarget organisms. Our previous study has shown that indoxacarb at 1 ~10 µM is less potent than its metabolite, DCJW, to block voltage-gated sodium channels in mammalian neurons. However, our knowledge of the mechanism of action of indoxacarb on insect sodium channels is very limited. The present study was undertaken to investigate the actions of indoxacarb and DCJW on tetrodotoxin-sensitive (TTX-S) sodium channels in cockroach thorathic ganglion neurons by the whole-cell patch clamp technique. DCJW at 0.1 ~ 1.0 µM slowly and irreversibly blocked TTX-S sodium channels. At -100 mV membrane holding potential, 100 nM DCJW reduced the peak amplitude of sodium current by 75.7 ± 12.1% (n = 6). Indoxacarb was less potent than DCJW. No changes in sodium channel activation kinetics was observed after application of indoxacarb or DCJW. Sodium channels of cockroach neurons undergo two kinetically distinct types of inactivation, fast inactivation and slow inactivation. DCJW at 100 nM shifted the half-maximum fast inactivation potential ($Vh_{0.5}$) to -72.1 ± 1.6 mV (n = 6) from the control value of -64.0 ± 4.0 mV (n = 6, $p < 0.01$) and shifted the $Vh_{0.5}$ of slow inactivation to -97.1 ± 3.4 mV from the control of -87.3 ± 3.2 mV (n = 3, $p < 0.05$). The voltage-gated potassium channel was not affected by indoxacarb or DCJW. These results suggest that the insect sodium channels are more sensitive to indoxacarb and DCJW than the mammalian sodium channels providing the basis for high insecticidal activity and low mammalian toxicity. Supported by NIH grant NS14143.

1578 INSECTICIDAL ACTIVITY OF ESSENTIAL OILS: OCTOPAMINERGIC SITES OF ACTION.

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A study was conducted to determine the insecticidal activity and mechanism of action of three essential oils (eugenol, a-terpineol and cinnamic alcohol) and an equal part mixture (3-blend) against American cockroaches (Periplaneta americana). To address species differences in response to treatment with the test oils, Carpenter ants (Camponotus pennsylvanicus De Geer), and German cockroaches (Blattella germanica) were included in this study. Exposed American cockroaches demonstrated hyperactivity followed by hyperextension of the legs and abdomen, then fast knockdown or quick immobilization followed by death. Ants and German cockroaches showed fast immobilization/knockdown followed by mortality. The 1:1:1 mixture (3-blend) was substantially effective against all test insects. One of the most remarkable observations was the increased frequency of heartbeats of American cockroaches in response to topical application of test oils. The changes in the pattern of cAMP level was biphasic. A significant increase in the cAMP level was found in response to 1 nmole/ml of eugenol, or 3-blend or 10 nmole/ml of a-terpineol. At higher concentrations a significant decrease in cAMP level was found. Blockage of octopamine receptors binding sites was also illustrated at lower concentrations of the test chemicals as judged by the decreased binding activity of 3H-octopamine to its receptors. In conclusion: 1- test oils are neuro-insecticides and their insecticidal activity is species-dependent, 2- a synergistic effect of the three oils was found when they were equally mixed (3-blend), and 3- octpaminergic system mediates the insecticidal activity of eugenol, a-terpienol and the 3-blend.

1579 DOSE CONVERSIONS BETWEEN UNITS OF MG/KG AND MG/M^2 FOR CYTOTOXIC ANTICANCER AGENTS: A CASE STUDY REGARDING THE IMPORTANCE OF CONSISTENT ESTIMATIONS OF BODY SURFACE AREA.

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The toxicity of cytotoxic anticancer drugs correlates more closely with body surface area (BSA) than with body weight (BW). Thus, it is standard practice to administer and compare such drugs on the basis of BSA. To interconvert between units of mg/kg and mg/m^2, it is necessary to know either BSA or BW. Accurate values for BW are easy to obtain. In contrast, BSA is difficult to measure, and, therefore, is usually calculated from a known BW. The relationship between BW and BSA is described by the formula $BSA(m^2) = b \times [BW(kg)]^{2/3}$, where b is a species-specific constant derived from empirical data. In practice, the values for b vary between laboratories (*e.g.*, values for b for the mouse have been found to vary from 0.090 to 0.096). Confusion and miscommunication can occur as a result of the use of different species-specific constants. For example, difficulties have arisen during the dose selection process for a novel cytotoxic anticancer agent with a steep dose-response curve. In this case, if different investigators use b values as varied as 0.099, 0.101, and 0.111, then animal BSA values for a 10 kg dog will vary by up to 12% (0.4631, 0.4724, and 0.5192 m^2, respectively). Also, although quantities of the anticancer drug are calculated from a target dose set in units of mg/m^2, the amounts of the drug given to the 10 kg dog will likewise vary, depending on the choice of b (0.926, 0.945, and 1.038 mg, respectively, for a 2 mg/m^2 dose). The use of a single set of species-specific constants by all investigators is proposed, as it would be beneficial in removing confusion from discussions of study designs and results. Such a proposal has an even more immediate benefit in the case of compounds with very steep dose-response curves, where even small variations in dosing can profoundly influence the results obtained and their interpretation.

1580 FOUR WEEK ORAL TOXICITY STUDY OF THE CANCER CHEMOPREVENTIVE AGENT RESVERATROL IN RATS.

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Resveratrol, a naturally occurring compound derived from grapes, inhibits cellular events associated with tumor initiation, promotion, and progression, and is being developed as a cancer chemopreventive agent. The present study examined the toxicity of resveratrol following 28 days of daily gavage administration to four groups of 20 rats/sex. Dose levels were 0, 300, 1000, and 3000 mg/kg/day, based on a two week pilot study. Oral administration of 3000 mg/kg/day resulted in nephrotoxicity observed as elevated serum BUN and creatinine levels, increased kidney weights, and gross and microscopic renal lesions. Dehydration at this dose was supported by reduced body weight gains (secondary to dec. food intake) and hyperalbuminemia. Additional clinical signs in high dose animals included labored breathing, hunched posture, decreased activity, rough coat, diarrhea, soft stool, and red material around the nose. Leukocytosis occurred in males, whereas anemia in females and possibly in males in the 3000 mg/kg/day dose group may have been a direct effect on RBCs (Inc.. total bilirubin), or secondary to renal injury (dec. erythropoietin synthesis). Increased serum ALT, ALKP and possibly total bilirubin indicated mild liver toxicity in the high dose group, but these changes were not supported histologically. At

this dose, liver QR, GST, UGT, and 2E1 were elevated, whereas 1A1 was slightly reduced. Effects seen at 1000 mg/kg/day included dehydration, labored breathing, reduced body weight gains and increased WBC count (males only). Dose-related plasma resveratrol levels in week 4 were relatively low given the high dose levels. On the basis of the present investigation, the no observed adverse effect level was 300 mg/kg/day. (Supported by NCI Contract No. NO1-CN-95132).

1581 PHARMACOKINETICS AND TISSUE DISTRIBUTION OF THE PROSTATE CANCER CHEMOPREVENTIVE AGENT LYCOPENE IN MALE BEAGLE DOGS.

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Lycopene, the predominant carotenoid in tomatoes and tomato products, has been associated with a decrease in prostate cancer risk. The present study was conducted to determine the pharmacokinetics and tissue distribution of lycopene in male dogs following 28 days of oral administration. Pilot studies examined the pharmacokinetics of lycopene following single doses of 10, 30 and 50 mg/kg, and 30 and 50 mg/kg/day for 4 consecutive days. The results indicated that the elimination half-life after a single dose of 10 to 50 mg/kg was about 36 hours, whereas the elimination half-life was somewhat shorter after four daily doses. In addition, peak plasma lycopene levels were similar following either 30 or 50 mg/kg/day for four days. Based on those results, 30 mg/kg/day was selected for the main study. An oral dose of 30 mg/kg/day administered to six dogs for 28 days resulted in steady-state plasma concentrations between 400 and 700 ng/mL 96 hours after the first dose. Mean apparent clearance, volume of distribution, and elimination half-life were 2.29 L/hr/kg, 96 L/kg, and 30.5 hours, respectively. Three dogs each were sacrificed one or five days after the last dose on day 29 and tissues were collected for lycopene analysis. Lycopene concentrations were highest in liver, adrenals, spleen, lymph node, and GI tract (in that order). Mean liver lycopene levels were 35 ug/g one day and 49 ug/g five days after cessation of treatment. Prostate lycopene levels were fairly low, with mean values less than 0.13 ug/g five days after dosing ceased (< 0.4% of liver levels). The long plasma elimination half-life was consistent with greater lycopene levels in tissues five days compared to one day following cessation of treatment. (Supported by NCI Contract No. NO1-CN-95055).

1582 DIETARY FOLATE DEPLETION IN BEAGLE DOGS: IS THYMIDYLATE SYNTHASE INHIBITOR TOXICITY INCREASED?

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The objective of the study was to determine if dietary folic acid depletion in beagle dogs altered the toxicity of the thymidylate synthase inhibitor (TSI), GS7904L (GW1843U89), currently under clinical development as an oncolytic. Classically, human cancer patients are deficient in nutrients, including Vitamins A and C and folic acid. An association has been made between patient's homocysteine status (a reflection of intracelullar folic acid)and enhancement of TSI toxicity. In previous studies with GS7904L, severe toxicity was observed in dogs treated with 2-3 mg/kg (40 – 60 mg/m2) as a single intravenous administration. To assess if GS7904L toxicity may be exacerbated in association with folic acid deficiency, a model was established to mimic this condition in beagle dogs. Two groups of young, female beagle dogs were fed either a standard certified diet or a diet deficient in folic acid (Teklad TD069) for 21 or 72 days. Both diets were equally palatable. Samples were collected over the timecourse of the study for serum folate, plasma homocysteine and whole blood hematology analyses. Clinical observations and body weights were recorded daily. All dogs were administered 1 mg/kg (20 mg/m2) GS7904L by bolus IV administration on either day 14 or day 66 of the study. At the time of drug administration, the serum folate levels were reduced 25 or 50% from baseline concentrations. Mild hematologic changes to white blood cell counts were noted in both the dogs fed the folate deficient and normal diets and no clinical toxicity was seen in any dog. These findings show that, in the dog under the conditions tested, dietary folic acid depletion did not increase GS7904L toxicity.

1583 EVALUATION OF THE ACUTE TOXICITY OF LIPOSOME-ENCAPSULATED VINCRISTINE SULFATE.

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Vincristine Sulfate Liposomes Injection (VSLI) utilizes sphingomyelin/cholesterol liposomes (55:45) as a systemic drug delivery vehicle for the treatment of neoplastic disease. In this presentation, the acute toxicity of VSLI was evaluated in Sprague-Dawley rats and beagle dogs. Rats (5/sex/dose) were administered single iv doses of 1.1 to 4.7 mg/m^2; dogs (1/sex/dose) were administered doses of 0.6 to 2.0 mg/m^2. Free VCR, empty liposomes, and saline groups were included in each study. In rats, VSLI was associated with dose-dependent body weight (BW) loss, myelosuppression, and testicular atrophy. There was no mortality among rats. Dogs treated with VSLI also showed dose-dependent BW loss and myelosuppression. Gastrointestinal toxicity was observed in both dogs administered VCR (1.1 mg/m^2; graded minimal in male, slight in female), in the male given VSLI at 1.1 mg/m^2 (minimal), and in animals given ≥1.6 mg/m^2 VSLI (minimal to slight). The only mortality was the male dog administered high-dose VSLI (2.0 mg/m^2). At 1.1 mg/m^2, BW loss in the free VCR dogs was 7% (male) and 22% (female) greater than in the VSLI-treated animals. The effects of equivalent doses of free VCR and VSLI on myelosuppression were comparable in rats (doses of 2.9 mg/m^2) and in dogs (doses of 1.1 mg/m^2) in terms of time of onset, severity, and duration. Free VCR and VSLI (1.1 mg/m^2) induced similar increases in aspartate aminotransferase levels in dogs, suggestive of muscle injury in the absence of increases in other aminotransferase levels. These data suggest that dogs may be more sensitive than rats to the toxic effects of VCR, whether free or liposomal, and that there are no new, unexpected acute toxicities observed for VSLI when compared to free VCR.

1584 A SINGLE DOSE INTRAVENOUS TOXICITY STUDY OF A LYMPHOTOXIN BETA RECEPTOR AGONIST ANTIBODY IN THE CYNOMOLOGUS MONKEY.

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LTBR mAb, a murine antibody with agonist activity toward the lymphotoxin beta receptor (LTBR), is currently being developed as a treatment for LTBR-expressing solid tumors. Agonism of the LTBR leads to cytokine release and death or differentiation of some neoplasms. A single intravenous injection of LTBR mAb in male cynomolgus monkeys at a dose level of 0.2 or 2.0 mg/kg had no effect on the study parameters. There were no abnormal clinical observations noted immediately following dose administration or over the subsequent 7- to 21-day observation period. Body weight changes and body temperatures recorded were within normal variations for cynomolgus monkeys. LTBR mAb had no effect on the delayed hypersensitivity (DTH) response or keyhole limpet hemocyanin (KLH) evaluations, as comparable variations were seen in all groups. The hematology, coagulation results, differential leukocyte counts, cellular morphology, and clinical chemistry parameters were unremarkable and similar among all groups on days 8 and 22. Serum concentrations of LTBR mAb at 2 hours post-dose were below the limit of quantitation in animals treated with 0.2 mg/kg LTBR mAb and were 41-52 µg/mL in animals treated with 2.0 mg/kg LTBR mAb. Antibodies to LTBR mAb were detected by day 8 in all LTBR mAb-treated animals. Findings noted at necropsy or identified during histopathologic examination were those known to occur as spontaneous changes in cynomolgus monkeys or associated with administration of Incomplete Freund's Adjuvant (used for DTH immunization). LTBR mAb administration did not alter the incidence or character of these findings. Based on the results of this study, a single intravenous administration of 2.0 mg/kg LTBR mAb was well tolerated.

1585 SAFETY OF A RIBOZYME TARGETING HER2 RECEPTOR mRNA (HERZYME ™) IN CD-1 MICE AND CYNOMOLGUS MONKEYS FOLLOWING DAILY BOLUS SUBCUTANEOUS INJECTIONS FOR 29 DAYS.

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A ribozyme (HERZYME) targeting the mRNA of the human epidermal growth factor receptor-2 (HER2) has been developed. Two toxicity studies were performed in CD-1 mice and cynomolgus monkeys. Animals received daily subcutaneous (SC) injections for 29 days of HERZYME (30, 100 or 300 mg/m^2/day). In CD-1 mice, HERZYME was well tolerated with no effects on clinical chemistry, hematology, viability, clinical observations, body weights, food consumption or gross pathology. HERZYME-related increases in liver weights were seen in mid- and high-dose females that resolved in the recovery groups. Cytoplasmic basophilia of renal cortical tubules and Bowman's capsule epithelial cells in the kidneys was observed for all HERZYME-treated groups and resolved in all but the high-dose group at recovery. In monkeys, there were no effects of HERZYME treatment on body weight, urinalysis, heart rate, blood pressure, ECG, clinical chemistry, clinical hematology or coagulation parameters. HERZYME-related changes in organ weights were: decreased thymus weights and slightly increased kidney weights in all treatment groups. The former was associated with microscopic findings of thymic atrophy and resolved completely in recovery animals. Accumulation of basophilic

granules in renal tubular epithelium and minimal to mild renal tubular degeneration was seen in most HERZYME-treated animals. Renal changes resolved in all males but persisted in one high-dose female. Kupffer cell hypertrophy and cytoplasmic basophilia was were observed in varying degrees in all HERZYME-treated animals. These changes had resolved in all but the high-dose animals. There were minimal injection site reactions in all HERZYME-treated monkeys that resolved in females and had partially resolved in males at recovery. In summary, daily SC administration of HERZYME for 29 days at doses up to 300 mg/m^2/day was well tolerated in mice and monkeys.

1586 28-DAY ORAL TOXICITY STUDY OF *Se*-METHYLSELENOCYSTEINE IN RATS.

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Se-Methylselenocysteine (MSC) is an organoselenium compound being developed for use in breast cancer chemoprevention. The present study was conducted to characterize the toxicity of subchronic oral administration of MSC in rats. CD rats (20/sex/group) received daily oral (gavage) administration of MSC at doses of 0, 0.5, 1.0, or 2.0 mg/kg body weight/day [target selenium doses of 0, 0.2, 0.4, or 0.8 mg/kg/day] for 28 consecutive days. Toxicology endpoints evaluated included body weight, food consumption, clinical observations, clinical chemistry, hematology, coagulation, ophthalmology, organ weights, gross pathology, and microscopic pathology. MSC induced a dose-related suppression of food consumption and body weight gain in both sexes; suppression of body weight gain was greater in females than in males. Administration of MSC at 2.0 mg/kg/day induced mild anemia and modest elevations in ALP, AST, ALT, and GGT in female rats; these changes were not seen in female rats receiving lower doses of MSC, nor in male rats at any dose level studied. At termination, dose-related hepatomegaly was present in both sexes. Dose-limiting histopathologic findings in male rats receiving MSC at 2.0 mg/kg/day included hepatocellular degeneration and arrested spermatogenesis; these changes were not observed in rats exposed to lower doses of MSC. Dose-limiting hepatocellular degeneration and atrophy of corpora lutea were present in female rats receiving MSC at doses of 2.0 and 1.0 mg/kg/day; hepatocellular degeneration of minimal severity was present in female rats receiving MSC at 0.5 mg/kg/day. These data suggest that the No Adverse Effect Level [NOAEL] for 28-day oral administration of MSC in male rats is 1.0 mg/kg/day. The NOAEL for 28-day oral administration of MSC in female rats is < 0.5 mg/kg/day. [Supported by contract N01-CN-05130 from the National Cancer Institute.]

1587 A 3 MONTH TOXICITY AND TOXICOKINETIC STUDY OF BAY 36-1677 (AN IL-4 SELECTIVE AGONIST) IN CYNOMOLGUS MONKEYS.

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BAY 36-1677 is a recombinant interleukin-4 selective agonist, a mutein of human IL-4 that selectively stimulates T and B cells with minimal endothelial cell activation. The purpose of this study was to evaluate the potential toxicity and toxicokinetics of BAY 36-1677 when administered daily (0-30 mcg/kg) or thrice weekly (100 mcg/kg) by subcutaneous (SC) injection to cynomolgus monkeys (6-8/sex/group) for 3 months. There were no BAY 36-1677-related adverse effects on clinical observations, food consumption, body weights, clinical chemistry, coagulation, urinalysis, blood pressure, heart rate, and electrocardiograms. Dose-dependent BAY 36-1677-associated effects consisted of increased white blood cell counts primarily attributed to lymphocytes. Flow cytometry analysis demonstrated increased B cells, T helper and T suppressor cells in most BAY 36-1677-treated animals. Microscopic evaluation revealed hypercellularity in the spleen and bone marrow, and increased periportal lymphoid cell infiltrates and leukocytic foci in the hepatic parenchyma. Injection sites showed cellular infiltrates in the subcutaneous tissue, perivascular dermatitis, and subcutaneous edema. Dose-related BAY 36-1677-specific antibodies were present at 2 months in all BAY 36-1677-treated animals. The antibody response was neutralizing with respect to BAY 36-1677 activity. Toxicokinetic data demonstrated that exposure was approximately proportional to the dose level and changes in toxicokinetics by 3 months correlated with the formation of antibodies. In conclusion, BAY 36-1677 was well tolerated in male and female cynomolgus monkeys with evidence of pharmacologic activity and no apparent signs of systemic toxicity, when administered daily up to 30 mcg/kg or three times weekly at 100 mcg/kg for a period of 3 months.

1588 A 13-WEEK TOXICITY STUDY OF BAY 16-9996 (AN IL-4 RECEPTOR ANTAGONIST) IN MALE AND FEMALE RHESUS MONKEYS WITH A 4-WEEK RECOVERY PERIOD.

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BAY 16-9996 is a recombinant human interleukin-4 receptor antagonist being developed for the treatment of severe asthma. It is designed to compete with endogeneous IL-4 for the binding of the IL-4 receptor, resulting in a switch from a Th2 cytokine secretion profile to a Th1 cytokine profile. To support ongoing clinical development, BAY 16-9996 was administered to male and female rhesus monkeys by subcutaneous (SC) injection every other day for 13 weeks followed by a 4-week recovery period at doses of 0, 0.3, 1.0 and 3.0 mg/kg (4-6/sex/group). BAY 16-9996 produced no apparent toxicity. There were no changes in clinical observations, food consumption, body weights, serum chemistry, hematology, coagulation, urinalysis, blood pressure, heart rate, body temperature, respiration, ophthalmic, or electrocardiographic parameters. BAY 16-9996-related findings were limited to injection site reactions: minimal to moderate perivascular infiltrates of mononuclear cells, and hemorrhage and fibrosis, which were largely reversible at the end of the recovery period. A dose dependent increase in the incidence of BAY 16-9996-specific antibodies was observed at three weeks post initiation of treatment. The magnitude of antibody response correlated with an increase in circulating levels of BAY 16-9996. In summary, BAY 16-9996 was well tolerated in male and female rhesus monkeys with no signs of systemic toxicity, when administered by SC injection every other day, at doses of up to 3.0 mg/kg for 13 weeks.

1589 3-MONTH INHALATION TOXICITY STUDY WITH sIL-4R IN CYNOMOLGUS MONKEYS.

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Immune function and general health were evaluated in cynomolgus monkeys administered human sIL-4R receptor *via* inhalation exposure (closed face mask system) or intravenous (IV) injection twice weekly for 12 weeks with a 12-week recovery period. Study animals were divided into four groups: placebo inhalation, low dose inhalation (0.1 mg/kg), high dose inhalation (1 mg/kg), or IV (0.2 mg/kg). Animals were evaluated for effects on clinical signs, body weights, ophthalmoscopy, hematology, clinical chemistry, urinalysis, pharmacokinetics and antibody response to the drug. Cellular immune function was assessed using delayed-type hypersensitivity (DTH) testing. Humoral immune function was assessed by measuring antibody response to keyhole limpet haemocyanin (KLH). Lymphocyte subpopulation analysis (CD3, CD4, CD8, CD14, CD20, CD56, and CD69) was conducted using flow cytometry of whole blood. All animals were subjected to a detailed necropsy, histological and immunohistochemical evaluations. There were no clinical signs, ophthalmoscopic findings or differences in body weight that could be attributed to administration of sIL-4R. Clinical pathology evaluation revealed no discernable treatment-related effects. Immune function was not affected by treatment with sIL-4R as assessed by DTH, antibody response to KLH, or by lymphocyte subset analysis. Antibody response was seen in 90% of animals receiving drug by inhalation; 20% of animals receiving drug IV. Exposure levels decreased with time in the inhalation-treated groups, most likely due to antibody development. Exposure levels were maintained in the IV-treated group. Terminal investigations did not reveal an effect of sIL-4R administration. Immunohistochemistry evaluations of lymphoid tissues revealed no differences in T and B lymphocyte populations. In conclusion, there was no evidence of systemic effects or evidence of alteration in immune function in animals administered human sIL-4R either by inhalation or IV for 12 weeks.

1590 DIFFERENTIAL SENSITIVITY OF 4 SPECIES TO OBSTRUCTIVE NEPHROPATHY PRODUCED BY AMDOXOVIR, A NUCLEOSIDE ANALOG WITH POTENT ANTIVIRAL ACTIVITY AGAINST HIV.

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1-β-D-2, 6-diaminopurine dioxolane (Amdoxovir, DAPD) is in Phase I/II clinical trials for treatment of HIV. Amdoxovir is rapidly absorbed after oral dosing and deaminated by adenosine deaminase to yield dioxolane guanosine (DXG), the active anti-HIV agent. One-month toxicology studies in mice and marmosets supported an IND. Amdoxovir produced obstructive nephropathy at doses ≧ 90 mg/kg in mice and 120 mg/kg in marmosets. One-month studies were also con-

ducted in rats and cynomolgus monkeys which were selected for chronic studies because, like humans, they produce more dilute urine. In rats, amdoxovir produced obstructive nephropathy at doses ≧ 400 mg/kg. In cynomolgus monkeys, a dose of 1200 mg/kg produced only minimal histopathologic alterations in the kidney. In chronic studies, obstructive nephropathy and sequellae was the only significant finding, occurring at doses ≧ 100 mg/kg in rats and 400 mg/kg in monkeys. The nephropathy was characterized by tubular dilatation, presence of crystalline material in tubular lumens, and degeneration, necrosis and regeneration of groups of tubules mainly in the cortex. There were no glomerular or vascular lesions. Both amdoxovir and DXG have limited solubility in aqueous media. Because the kidney is the major organ of elimination, it is hypothesized that amdoxovir or DXG precipitate in kidney tubules as urine is concentrated. In summary, similarly characterized obstructive nephropathy was observed in subchronic studies of amdoxovir in 4 species and in chronic studies in 2 species. However, while renal tubular obstruction occurred at lower doses in small, water-conserving mice and marmosets, higher doses were required to produce obstruction in less water-conserving rats and cynomolgus monkeys. Based on water conservation levels, it is anticipated that the dose level (per kg basis) causing obstructive nephropathy in humans will be similar to that in cynomolgus monkeys. Thus, at doses selected for clinical trials, the risk of obstructive nephropathy appears low.

1591 PHARMACOKINETIC DISPOSITION IN ARTHROPATHY OF JUVENILE DOGS INDUCED BY A QUINOLONE ANTIMICROBIAL AGENT.

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Quinolone antimicrobial agents including ofloxacin induce arthropathy in juvenile animals, but not in adult animals. To examine the age-specific difference in susceptibility to ofloxacin arthropathy, its pharmacokinetic disposition and early pathological change in the articular cartilages were compared between male juvenile (3-month-old) and adult (18-month-old) dogs. Ofloxacin was orally administered at 5, 10 or 20 mg/kg/day to these dogs for 8 consecutive days, and serum and cartilage drug concentrations were periodically analyzed by HPLC. Serum Cmax and AUC0-24h, and drug concentrations in the cartilage of juvenile dogs were increased in a dose dependent manner. At 20 mg/kg/day, the drug disposition of juvenile dogs was equal to or lower than those of adult dogs. Macroscopically, arthropathy characterized by fluid-filled vesicles in the articular surface was observed only in the juvenile dogs receiving 10 and 20 mg/kg/day, but not 5 mg/kg/day. Microscopically, the juvenile dogs showed cavity formation with unmasked collagen fibers in the middle zone of the articular cartilage and numerous degenerative or necrotic chondrocytes around the cavity on day 2, followed by appearance of chondrocytes clusters on day 8. Meanwhile, the adult dogs represent the thin and zonal different cartilage layer, in which the typical tidemark was observed, suggesting that the existence of the mature articular cartilage tissue may related to protective action on arthropathy. Based on these results, high susceptibility to arthroptahy in juvenile dogs can not be explained only by drug disposition to the articular cartilage. The interaction between ofloxacin and immature cartilages on cell cycles and matrix synthesis may be important for pathogenesis of arthropathy.

1592 REPEAT-DOSE TOXICITY STUDIES IN RATS WITH TWO SUSTAINED RELEASE FORMULATIONS OF LY307161, A PROTEASE-RESISTANT GLP-1 ANALOG.

R. A. Byrd and K. F. Mace. *Toxicology and Drug Disposition, Eli Lilly and Company, Greenfield, IN.* Sponsor: P. Sausen.

LY307161 is a protease-resistant analog of Glucagon-Like Peptide 1 (GLP-1) that is under development for treatment of Type 2 diabetes. Two sustained release formulations of LY307161, designated NDP-1 or NDP-2, were evaluated in separate 1-month rat studies. F344 rats (8-12/sex/group) were given daily subcutaneous doses of 0, 1, 4, or 20 mg/kg. GLP-1 pharmacodynamic effects observed with NDP-1 included decreases in body weight (↓5-16%), food consumption (↓6-16%), and triglycerides (↓9-44%) at all doses. No target organ toxicity was identified with NDP-1. Toxicologically important findings with NDP-1 were limited to injection site reactions in the 4- and 20-mg/kg groups characterized by swelling, minimal-to-marked granulomatous inflammation, and correlative changes in clinical pathology consistent with the inflammation. Injection site reactions in the 1-mg/kg group were of lesser incidence and magnitude, were not associated with clinical pathology changes, and were not clinically apparent. On Day 29, $AUC_{0\text{-}24hr}$ values for the 1-, 4-, and 20-mg/kg groups were 232, 843, and 2649 ng•hr/mL, respectively. With NDP-2, GLP-1 pharmacodynamic effects also included decreases in body weight (↓7-14%), food consumption (↓7-18%), and triglycerides (↓17-39%); no target organ toxicity was identified with NDP-2. Injection site reactions with NDP-2 occurred only in the 4- and 20-mg/kg groups without clinical pathology changes, and the severity of the reactions, characterized as minimal-to-moderate, was reduced relative to NDP-1. Plasma exposure with NDP-2 was higher relative to NDP-1; the $AUC_{0\text{-}24hr}$ values on Day 33 for the 1-, 4-, and 20 mg/kg groups were 380, 1247, and 4424 ng•hr/mL, respectively. In conclusion, NDP-2 exhibited improvements in local tolerability and bioavailability relative to NDP-1 in rats. The NOEL for target organ toxicity with either LY307161 formulation was 20 mg/kg. Plasma exposure (AUC) at the NOEL was 284- to 449-fold the maximum anticipated human exposure thus demonstrating an adequate Margin of Safety.

1593 REPEAT-DOSE TOXICITY STUDIES IN DOGS WITH TWO SUSTAINED RELEASE FORMULATIONS OF LY307161, A PROTEASE-RESISTANT GLP-1 ANALOG.

K. F. Mace and R. A. Byrd. *Toxicology and Drug Disposition, Eli Lilly and Company, Greenfield, IN.* Sponsor: P. Sausen.

LY307161 is a protease-resistant analog of Glucagon-Like Peptide 1 (GLP-1) that is under development for treatment of Type 2 diabetes. Two sustained release formulations of LY307161, designated NDP-1 or NDP-2, were evaluated in separate 1-month dog studies. Beagle dogs (3/sex/group) were given daily subcutaneous doses of 0, 0.2, 0.8, or 3 mg/kg. GLP-1 pharmacodynamic effects observed with NDP-1 included vomiting, decreased body weight (↓1-7%), and decreased qualitative food consumption at all doses. No target organ toxicity was identified with NDP-1. Toxicologically important findings in the 0.8- and 3-mg/kg groups were limited to injection site reactions characterized by swelling, moderate-to-marked pyogranulomatous inflammation, and correlative changes in clinical pathology consistent with the inflammation. Injection site reactions in the 0.2-mg/kg group were of lesser magnitude, were not associated with important clinical pathology changes, and were not clinically apparent. On Day 29, $AUC_{0\text{-}24hr}$ values for the 0.2-, 0.8-, and 3-mg/kg groups were 71, 210, and 620 ng•hr/mL. With NDP-2, GLP-1 pharmacodynamic effects also included vomiting, decreased body weight (↓3-9%), and food consumption (↓1-23%) at all doses; no target organ toxicity was identified with NDP-2. Toxicologically important findings were again limited to injection site reactions at all NDP-2 doses, but the severity of the reactions, characterized as minimal-to-moderate pyogranulomatous inflammation, was reduced relative to the NDP-1 formulation. Correlative clinical pathology changes were also diminished relative to NDP-1. On Day 28, $AUC_{0\text{-}24hr}$ values for the 0.2-, 0.8-, and 3-mg/kg groups were 52, 137, and 349 ng•hr/mL. In conclusion, LY307161 NDP-2 exhibited an improved local tolerability profile in dogs. The NOEL for target organ toxicity with either LY307161 formulation was 3 mg/kg. Plasma exposure (AUC) at the NOEL was 35- to 63-fold the maximum anticipated human exposure thus demonstrating an adequate Margin of Safety.

1594 HEPATIC PEROXISOMAL PROLIFERATION AND MICROSOMAL ENZYME INDUCTION BY THE LIPID-REGULATING AGENT CI-1027 IN RODENT AND NONRODENT.

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CI-1027 (6, 6'-oxybis[2, 2-dimethyl-4-hexanoic acid]-Ca) is a dicarboxylic acid lipid-regulating agent in experimental models of dyslipidemia. Hepatic peroxisome proliferation and microsomal enzyme induction were evaluated after oral CI-1027 administration for 2 weeks in beagle dogs, 4 weeks in Wistar rats and cynomolgus monkeys, and 13 weeks in B6C3F1 mice. Peroxisomal enzyme activity markers were carnitine acetyltransferase, acyl CoA oxidase, β-oxidation, and catalase, and microsomal enzyme activity markers included lauric acid hydroxylase, an activity marker for CYP4A1, and UDP glucuronyl transferase (GT). No hepatic changes occurred in dogs given doses up to 1000 mg/kg. In monkeys, liver weight increased at 300 mg/kg, but was unchanged at 10 to 100 mg/kg. However, there was no pattern of enzyme changes indicative of peroxisomal proliferation or microsomal enzyme induction in monkeys. In rats at all doses, 30 to 750 mg/kg, liver weight increased up to ~100% and peroxisomal enzyme activities up to 6-fold. Lauric acid hydroxylase increased up to 11-fold at all doses in rats and GT activity increased up to 1.5-fold at 100 to 750 mg/kg. Increased CYP4A concentrations were confirmed with an ELISA. In mice, liver weight increased up to 25%, peroxisomal enzyme activities increased 48% to 4.7-fold, and microsomal lauric acid hydoxylase increased 3- to 10-fold at all doses (500, 1000, and 2000 mg/kg). Peroxisome proliferation was confirmed by ultrastructural evaluation. Toxicokinetic profiles revealed that CI-1027 exposures were less than dose-proportional in all species. It is concluded that CI-1027 is similar to related compounds that induce hepatic peroxisome proliferation and microsomal enzyme induction in rats and mice, but not in nonrodent species.

1595 PRECLINICAL SAFETY EVALUATION OF THE LIPID-REGULATING CANDIDATE CI-1027 IN CYNOMOLGUS MONKEYS AFTER 4 AND 26 WEEKS OF ORAL DOSING.

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The sub-acute and chronic effects of CI-1027 (6, 6'-oxybis[2, 2-dimethyl-4-hexanoic acid]-Ca), a peroxisome proliferator in rodents, were investigated in male and female cynomolgus monkeys receiving 10, 30, 100, or 300 mg/kg daily by gavage for 4 weeks, or 10, 30, or 100 mg/kg for 26 weeks. In the 4-week study, 1 male and 2 females at 300 mg/kg were euthanized moribund during Week 3 or 4. Clinical laboratory changes consisted of decreased RBC count/hematocrit/ hemoglobin in females at 100 and 300 mg/kg (10% to 27% from pretest), and increased APTT (41% to 72%) and PT (14%) in males at 100 and 300 mg/kg. Pathological changes included increased relative liver weight at 300 mg/kg (58% to 93%), hepatocellular hypertrophy and/or hepatocellular fatty change, and decreased fine vacuolation of the adrenocortical zona fasciculata at 100 and 300 mg/kg. There was no hepatic microsomal or peroxisomal enzyme induction, indicating absence of peroxisome proliferation. In the 26-week study, RBC count/hematocrit/hemoglobin were 11% to 18% lower in males and females at 100 mg/kg. Slight decreases in mean corpuscular volume (7.1%) and mean corpuscular hemoglobin (9.2%) were also noted in males at 100 mg/kg. Increase in absolute (32% to 34%) and relative (46% to 49%,) liver weight and relative kidney weight (33% to 45.6%) were noted in males and females receiving 100 mg/kg. Microscopic findings consisted of a dose-related increase in the relative incidence and severity of lipid vacuolation in the liver of all males and females receiving 30 and 100 mg/kg. Lipid vacuolation was also noted in the kidneys of 2 animals at 30 mg/kg, and all animals at 100 mg/kg. CI-1027 appears to act similar to other peroxisome proliferators in primates.

1596 TOXICOKINETIC-TOXICODYNAMIC RELATIONSHIPS IN HUMAN PHENOBARBITAL POISONINGS.

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The value of PK-PD relationships in clinical pharmacology is now well recognized. However, the potential interest of Toxicodynamic-Toxicokinetic (TK-TD) relationships in medical toxicology has been poorly investigated. The aim of this study was to correlate the depth of coma with plasma phenobarbital (PB) concentrations. Materials and Methods: Plasma PB concentrations were measured using an enzymatic assay. The depth of coma was assessed using the Glasgow coma scale (GCS). Non-linear regression was used for modeling TK-TD relationships. Results : TK-TD relationships were studied in 6 acute PB poisonings. Two patients were previously treated with PB. Previous treatment was unknown in 2 patients. Mixed drug poisoning was noted in 2 patients. The GCS at the time of hospital admission was 3 in the 6 patients, the mean plasma PB concentration was 710.5 +/- 281.9 µmol/l (range: 360 – 1030). The TK-TD relationship was well fitted with the sigmoidal Emax model. In the 6 patients, the mean Hill coefficient (m+ SD) was 6.0 +/- 1.9 and the mean C50 was 289.7 +/- 97.1 µmol/l (range: 170 – 440). Discussion: A maximal toxic effect (GCS of 3) was associated with a wide range of plasma PB concentrations. During the course of PB poisoning, the relationship between the depth of coma and the corresponding plasma PB oncentration is of a sigmoidal shape. The high value of the Hill coefficient showed that a small decrease in plasma PB concentrations near the C50 was associated with a dramatic improvement in their level of consciousness. In the 6 patients, the mean C50 was approximately equal to 3-fold the upper limit of the therapeutic plasma concentration of PB given by our toxicological laboratory (< 100 µmol/l).

1597 CLINICAL SYNDROME ASSOCIATED WITH ALPRAZOLAM INGESTION IN DOGS: 238 REPORTS (JANUARY 1998-AUGUST 2001).

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Alprazolam is a benzodiazepine that is used as an anti-anxiety agent. Alprazolam acts at the limbic, thalamic, and hypothalamic level of the CNS and has anxiolytic, sedative, hypnotic, skeletal muscle relaxant, and anticonvulsant properties. A retrospective study was conducted of alprazolam ingestion in dogs that were reported to the ASPCA Animal Poison Control Center between January 1998 to August 2000. Data analysis included amount ingested, clinical effects, and time of onset of signs. Two hundred and thirty-eight cases of suspected alprazolam toxicoses in dogs (ranging in age from 11 weeks to 13.5 years) were evaluated. Approximate ingested dosages ranged from 0.01 to 5.55 mg/kg. Of the two hundred and thirty-eight cases of suspected toxicosis, there were 108 males (45%) and 113 females (47%.) Gender information was not available in 17 dogs (7%.) Incidence of clinical signs reported included: ataxia/disorientation (68 %, 161 cases), depression (34%, 83 cases), hyperactivity (13%, 30 cases), vomiting (8%, 19 cases), weakness (7%, 18 cases), tremors (5 %, 13 cases), and vocalization (4%, 10 cases.) Other signs reported included tachycardia, tachypnea, hypothermia, diarrhea, and increased salivation. In 38% of cases, clinical signs developed within 10 – 30 minutes. Treatment of overdoses would include standard decontamination procedures, such as emesis and activated charcoal. Flumazenil is a specific benzodiazepine antagonist and could be used to treat dogs with severe CNS depression.

1598 ATTENUATION OF HEPATIC FIBROSIS OBSERVED WITH A "GUTLESS" ADENOVIRAL VECTOR.

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Adenoviral vectors are being explored as potential therapeutic agents, although clinical utility has been limited due to the adverse effects seen in liver following intravenous injection. In previous studies, we demonstrated in male monkeys and CD-1 mice that an early generation adenoviral vector caused hepatocellular apoptosis and activation of hepatic stellate cells with subsequent fibrosis. In this study, in order to determine if viral gene expression contributes to hepatic toxicity, male and female CD-1 mice were injected with 6×10^{11} (low dose), 3×10^{12} (mid dose) or 1.5×10^{13} (high dose) viral particles/kg of either an early generation vector (Av3Null) or a "gutless" vector with all viral genes removed (AGVNull). Serum IL-6 levels were measured during the first week after dosing. Selected clinical pathology parameters were evaluated on days 2, 8, 15, 29, 43, and 57. Histopathology was evaluated at day 8 and 57. Dramatic increases (200 fold) in serum IL-6 levels were seen in high dose mice 6 hours after receiving either vector. Significant elevations in serum liver enzymes were seen in high dose mice with either vector on day 2, with a decrease in platelets observed in these animals at day 4. Values returned to normal by the termination of the study. Morphological changes were present following treatment with either vector and included hepatocellular apoptosis, extramedullary hematopoiesis, activation of stellate cells and increased extracellular matrix production. Hepatic fibrosis (as measured by sirius red index) in both sexes was not significantly different from controls following treatment with the gutless vector, but significant differences were seen in Av3Null-treated animals. In summary, liver changes produced by the gutless vector were less severe than those due to Av3Null. Since serum liver enzyme measurements did not reveal these vector-induced hepatic changes, studies are currently underway to determine if serum collagen and alpha-GST levels can be used as markers for early detection of hepatic injury and fibrosis.

1599 BIODISTRIBUTION STUDIES AS PART OF THE PRECLINICAL TOXICOLOGICAL ASSESSMENT OF CANARYPOX GENE TRANSFER VECTORS.

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One critical point in the non-clinical safety evaluation of gene transfer products is the evaluation of the distribution of the exogenous genetic material after administration. Therefore, biodistribution studies are requested by the authorities in the early development phases. The goals of vector biodistribution studies are: (1) to assess possible dissemination of the vector to the germ-line cells with the potential risk of transmission to the offspring and (2) to assess dissemination to non-target tissues, providing information about the potential target organs for toxicity, which helps with the design and conduct of toxicology studies. As part of a toxicology study, we evaluated the biodistribution of the canarypox virus DNA, ALVAC(2)-gp100 mod vaccine, by nested qualitative PCR in Cynomolgus monkey tissues following repeated administrations by the intranodal or subcutaneous route. Forty days after the last ALVAC injection, ALVAC(2)-gp 100 mod DNA sequences were found by PCR in the injected inguinal lymph node in 4 out of 4 intranodal-injected animals, and in the right axillary lymph node (draining the last injection site) in one out of 4 subcutaneous-injected animals. There were no positive PCR results in any other organs, including gonads. These results complete those from a generic study in the rat with an « empty » ALVAC vector (no inserted gene) administered by 3 different routes (intravenous, subcutaneous and intramuscular). This generic study included several sacrifice time points and employed a quantitative PCR method. This strategy, employing an extensive analysis of the vector itself without any inserted genes together with PCR data on the candidate product as part of the pivotal toxicology study, not only complies with both US and European requirements but also represents the most rational approach in terms of cost and analysis.

1600 LYSOSOMAL-STORAGE DISORDER INDUCED BY ELMIRON IN RATS AND MICE.

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Elmiron, a highly sulfated, semi-synthetic pentose polysaccharide with properties similar to heparin, is used for the treatment of interstitial cystitis. Thirteen-week gavage studies were conducted by administering the drug in deionized water to F344/N rats and B6C3F1 mice once daily, 5 days per week for up to 13 consecutive weeks, at doses of 0, 63, 125, 250, 500, and 1000 mg/kg body weight. No significant drug-related effects were observed in body weight, survival, and clinical and gross necropsy results. Significant organ weight increases were seen in the liver, lung, and spleen of both species and the kidney of rats, mainly in groups treated with 250 mg/kg/day and above. Hematological analysis indicated increases for both species in the white blood cell and lymphocyte counts. Sites of toxicity identified histopathologically were the rectum, liver, mesenteric and mandibular lymph nodes (both species), spleen (mice only), and lungs and kidneys (rats only). Lesions consisted mainly of infiltration into multiple tissues of vacuolated histiocytes; histochemical investigation indicated the presence of neutral and acidic mucins and lipidic material within the vacuoles. Transmission electron microscopy identified these vacuoles as lysosomal structures that exhibited a variety of contents. On the basis of our findings, we propose that Elmiron was absorbed through the focally disrupted rectal mucosa, accumulated in the lamina propria, phagocytized, and then transported *via* the lymphatics and blood to the various organ sites manifesting histiocytic infiltration. The cytoplasmic membrane-bound structures within macrophages are lysosomes containing membranous material of cellular origin and, perhaps, remnants of the phagocytized test material, Elmiron.

1601 A FRACTURE HEALING STUDY OF PRINOMASTAT™ IN THE SKELETALLY MATURE SPRAGUE-DAWLEY RAT.

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This study was designed to investigate the effect of prinomastat (a matrix metalloproteinase inhibitor) on fracture healing in the rat, during twice daily oral administration at dose levels of 20, 100 and 250 mg/kg/day for a minimum of 3 or 6 consecutive weeks, commencing the day following surgical induction of a closed femoral fracture with intramedullary pinning. Selected endpoints (histopathology, biomechanical testing, *ex vivo* peripheral quantitative computed tomography (pQCT) and *ex vivo* dual energy X-ray absorptiometry (DXA) provided an assessment of effects on the reparative and modelling/remodelling phases of the healing process. Surgery involved a parapatellar incision into the joint capsule of anesthetized rats, lateral retraction of the patellar ligament and insertion of an intramedullary Kirshner wire into the femur such that knee joint mobility was unaffected. Following wound closure, a closed transverse fracture of the femoral diaphysis was created using a blunt guillotine driven by a dropped weight. Radiographs were taken following surgery. Minimal comminution and angulation of the intramedullary pin was permitted. Prinomastat was well tolerated, with no adverse toxicological effects, although a reduction in body weight gain was noted. Treatment resulted in reduced biomechanical strength associated with reduced callus formation, evidenced by a reduction in callus area and mineral content as determined by DXA and/or pQCT. Histopathological data suggested prinomastat delayed callus formation/remodelling in rats with an experimentally induced closed fracture. These data suggest that prinomastat delays fracture healing in rats, as evidenced by a reduced callus and biomechanical properties at Week 3 and by persistent soft callus at Week 6, however, prinomastat does not completely inhibit the healing process.

1602 POSTPRANDIAL SERUM CALCIUM IS A SURROGATE MARKER FOR CHANGES IN INTESTINAL CALCIUM ABSORPTION INDUCED BY THE VITAMIN D ANALOG RO 65-2299 IN THE DOG.

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Calcium-related toxicity, typical for the class of vitamin D (VitD) analogs, is characterized by increased intestinal calcium (Ca) absorption accompanied by increased renal Ca excretion and at an advanced stage of disturbed Ca homeostasis, hypercalcemia. While urinary Ca and serum Ca concentrations can be easily measured by standard methods, quantification of intestinal Ca absorption requires specialized methods not normally applied in routine studies. In toxicity studies with the VitD analog Ro 65-2299, we established a simple indirect method to identify changes in intestinal Ca absorption in the dog. The animals were adapted to a defined amount of feed given during one hour period in the morning. Ro 65-2299 was administered daily by the oral route during this hour. After at least 5 days treatment, blood was collected before and at 6-8 time points within 6-7 hours after dosing. We demonstrated a dose-dependent effect on the increase in serum Ca concentration during the first few hours after feeding. This was well correlated with Ca-related toxicity observed in subchronic 4-week and 13-week toxicity studies. At non-toxic doses up to 100 mg/kg/day, the postprandial serum Ca concentrations did not exceed the normal range of 2.4-3.0 mmol/l. At 200 mg/kg/day the postprandial Ca level rose slightly above the normal range in some animals but returned to normal before the next day feeding. The 200 mg/kg/day caused only a transient hypercalciuria only after 5 to 8 weeks of treatment. At higher doses (300 and 400 mg/kg/day over 5 days; 250 mg/kg/day over 4 weeks) postprandial calcium levels markedly exceeded the normal range, which corresponded with severe hypercaliuria and hypercalcemia. In conclusion, the time-concentration profile of serum Ca after feeding under defined conditions is considered a surrogate marker for changes in intestinal Ca absorption, due to treatment with VitD analogs.

1603 ABSENCE OF TOXICOLOGICAL EFFECTS FROM THE IMMUNOLOGICAL ADJUVANT MONOPHOSPHORYL LIPID A (MPL®).

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The addition of the immunostimulatory adjuvant monophosphoryl lipid A (MPL®), a detoxified bacterial lipopolysaccharide, to a range of conventional vaccine (eg malaria, herpes, hepatitis) and allergy vaccine (eg grass pollen) materials is the subject of ongoing clinical trials. However despite this use, there are no robust published data on the nonclinical safety assessment of MPL®. Thus a series of preclinical studies have been performed with the material to examine for potential toxicological safety issues. An intravenous cardiovascular/respiratory function assessment in anaesthetized dog (at up to 100 µg/kg/day) showed no adverse effects. Repeat dose subcutaneous or intravenous investigations in the rat (for up to 4 weeks and at up to 2500 µg/kg/day), rabbit (once weekly for 8 weeks at up to 200 µg/kg/occasion) and dog (for up to 2 weeks and at up to 1200 µg/kg/day) showed no unexplained study findings. Expected immunostimulatory effects and/or signs of toxicity associated with over-stimulation of the immune system (notably increase spleen and white cell activity) were seen in the rat and dog. Embryo-fetal and peri-post natal subcutaneous reproduction toxicity studies in rat and rabbit (at up to 100 µg/kg/day) showed no adverse effects. Genotoxicity studies (Ames test and chromosome aberration assay) were negative. Overall, the lack of findings of toxicological concern support the hypothesis that MPL® is a safe adjuvant for human use.

1604 SB-242235: ORAL MALE FERTILITY STUDY IN RATS.

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SB-242235 is a selective p38 protein kinase inhibitor which suppresses pro-inflammatory cytokine biosynthesis. The purpose of this study was to evaluate the potential effects of SB-242235 when administered orally by gavage to male rats, on mating, fertility and gonadal function. Male Sprague-Dawley rats (25/group) were treated with 1% methylcellulose or 3, 10 or 100 mg/kg/day of SB-242235 for 85 to 89 days. On days 15 to 22 and on days 71 to 78, the males were cohabited with untreated, virgin female (F0) rats. Mated females were killed on day 20 postcoitus. Drug-related mortality (1 of 25 males) and clinical signs (salivation, stereotypic, kneading behavior) were evident at 100 mg/kg/day. Overall, body weight gain for males was reduced (13%, 19%, 46%, at 3, 10, 100 mg/kg/day, respectively) as was food consumption (up to 8%, 22% at 10, 100 mg/kg/day, respectively). There was no effect on pregnancy following mating on days 15 to 22, but pregnancy rate was significantly decreased *vs.* controls (4% *vs.* 96%) for males given 100 mg/kg/day and mated on days 71 to 78. At 100 mg/kg/day, decreases were evident in weights of the epididymides (left epididymis, intact (22%), right caput and corpus (10%) and right cauda epididymis (36%), and ventral prostate (34%), number of spermatids/gram testis (25%) and sperm/gram cauda epididymis (52%). There was a decrease (83%, control vs 7%, 100 mg/kg/day) in the percentage of motile sperm. In conclusion, SB-242235 produced mortality at 100 mg/kg/day and overt toxicity at all doses. Reproductive toxicity was demonstrated by reduced organ weights (epididymides and ventral prostate), impaired gonadal function (reduced spermatogenesis and sperm motility) and infertility (reduced number of litters sired) among males given 100 mg/kg/day for 10 weeks.

1605 CYCLOSPORIN AND RITONAVIR ARE POTENT P-GP INHIBITORS *IN VITRO*.

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In humans the multidrug resistance (MDR-1) gene codes p-glycoprotein (P-gp). As an ATP-dependent export pump, P-gp is involved in the transport of hydrophobic, neutral or cationic xenobiotics in a variety of tissues. In addition, the sister of P-glycoprotein (SPGP) is the major canalicular bile salt export pump of mammalian liver. Certain drugs, as for example, cyclosporine (CSA), have been shown to inhibit both P-gp and SPGP, which can result in an impairment of bile flow (cholestasis). Protein trafficking from Golgi to the apical plasma membrane is an important step in the maturation of P-gp. In this study, we compared chrysin, ritonavir, progesterone, prazosin, estradiol-17beta-glucoronide, CSA, and wortmannin, as inhibitors of P-gp in two cell lines based on competition with the efflux of a fluorescent P-gp probe, calcein. The inhibition of P-gp efflux function was investigated following drug treatment in a cell-based assay. The assay compared Madin-Darby canine kidney (MDCK) cells and MDCK cells over-expressing the transfected human MDR1 gene. In the absence of transport inhibition, it has previously been shown that intracellular retention of calcein is high in MDCK cells but low in the MDR1 cells. As a result, we observed inhibition of P-gp efflux function (i.e., increased intracellular calcein fluorescence) by CSA and ritonavir. Our results provide a mechanism for clinically observed effects of CSA and ritonavir.

1606 MISOPROSTOL HAS POTENT *IN VITRO* TUMORICIDAL ACTIVITY THAT IS INDEPENDENT OF ITS PROSTANOID ACTIVITY.

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Misoprostol (MP) was originally developed as a synthetic prostaglandin (PG) agonist for treating gastric ulcers caused by non-steroidal anti-inflammatory drugs. As part of our research program on the immunomodulatory effects of prostanoid compounds, we investigated the effects of MP on the growth and viability of immune-related tumour cell lines. Mouse leukaemia P388 cells were exposed *in vitro* to MP over 48 hours, and tumouricidal activity was measured by changes in cell number and viability (*via* trypan blue exclusion). MP was potently tumouricidal to P388 leukaemia cells, with a 50% inhibitory concentration (IC_{50}) of 100 nM - exhibiting a similar dose-response curve as that reported for MP's immunosuppression of mouse thymocyte proliferation [1]. In contrast, PGE_2 (the mammalian PGE form and PG receptor agonist) and dibutyryl cyclic AMP (a stable membrane-permeable analog of the intracellular PG messenger, cAMP), had minimal effects on P388 cells, even when used at >0.1 mM, which is orders of magnitude greater than the IC_{50} values for their immunosuppressive effects [1]. Furthermore, MP was equally cytotoxic to the adriamycin-resistant sub-line of P388 (P388/ADR), and was generally more toxic to immune-related tumour cell lines, with the following order of potency: P388 = P388/ADR > mouse T-lymphoma (Yac-1) > mouse mastocytoma (P815) > mouse fibrosarcoma (WEHI-164). This study indicates that MP is potently cytotoxic to leukaemia cells, in a manner that is independent of both its prostanoid activity and the mechanisms involved with tumour resistance to adriamycin. These findings have important implications for the potential use of MP as a selective chemotherapy of immune-related tumours. [1] D R Haynes, M W Whitehouse and B Vernon-Roberts (1992) Immunology, 76: 251-257.

1607 AN INTEGRATED APPROACH TO HAZARD EVALUATION FOR WORKER SAFETY ASSESSMENT OF PHARMACEUTICALS AND SYNTHETIC INTERMEDIATES.

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Workplaces in the pharmaceutical industry are unique — hazards may be posed both by synthetic chemistry intermediates and the biologically active substances (drugs) created as products. Effective occupational health programs in the pharma industry rely on substance hazard profiles. These are used to identify appropriate containment and protective strategies and are created/updated to support the process of drug development. However, hazard characterization is constrained by cost containment efforts, ethical considerations in use of laboratory animals, and recognition of high rates of attrition of substances in development. We have developed an animal-sparing, tiered approach for hazard characterization of drugs and intermediates aligned with the development cycle. An initial tier includes physical-chemical characterization (pH/partition coefficient), computerized QSAR (DEREK), *ex vivo* assays assessing skin and eye irritant potential (transcutaneous electrical resistance (TER) and rabbit enucleated eye (REET) tests), high-throughput mutagenicity assays (SOS*umu*, HTFT, COMET) and *in vitro* cytotoxicity tests (predicting acute lethality). Screening of pharmaceuticals occurs earlier in the development cycle than that for intermediates, accounting for changes in the identity of intermediates as syntheses are scaled up for manufacture. A second tier of evaluation includes the local lymph node assay (LLNA) and conventional assays for genetic toxicity (Ames, L5178Ytk+/-). The initial *in silico, in vitro,* and *ex vivo* screens aid by eliminating substances causing positive results from subsequent *in vivo* evaluation for acute oral toxicity, dermal/ocular irritation, dermal sensitization, and potential target organ toxicity (final tier). Benefits of this approach include support for data-based judgments regarding substance hazards and required labeling at appropriate points in development, and the inherent potential for minimizing extensive *in vivo* testing.

1608 NEUROTOXICOLOGY OF BLOOD-BRAIN BARRIER AND BLOOD-CSF BARRIER: STRUCTURAL AND FUNCTIONAL CONCERNS.

W. Zheng[1] and M. Aschner[2]. [1]*Environmental Health Sciences, Columbia University, New York, NY and* [2]*Physiology & Pharmacology, Wake Forest University, Winston-Salem, NC.*

To enter the brain parenchyma chemicals have to cross the brain barrier systems that safeguard the milieu of the central nervous system (CNS). The barrier that separates the systemic circulation from the brainÕs interstitial fluid is defined as the blood-brain barrier (BBB), while the one that separates the systemic circulation from the cerebrospinal fluid (CSF) is named blood-CSF barrier (BCB). Though ubiquitous, certain areas of the brain, referred to as the circumventricular areas are void of the restrictive properties that characterize the BBB. Research in the past decade suggests that beyond the well-defined role as ÒgateÓkeepersÓ, the brain barriers actively participate in brain ontogenesis and development, neuroendocrine regulation, drug efflux and metabolism, aging processes, chemical-induced neurotoxicities, and the etiology of a number of neurodegenerative diseases. For example, the interaction of manganese (Mn) with iron (Fe) metabolism at brain barrier interfaces alters the cerebral homeostasis of Fe, which may contribute to Mn-induced neurodegenerative disorder. Exposure to organophosphate insecticides impairs the BBB, leading to neurotoxic consequences. Metabolism and/or efflux of drugs and endogenous molecules at the barriers are not only critical for an effective drug therapy, but also essential in preventing CNS side effects. Cumulative evidence also suggests that the BBB, by partaking in the development of cerebral amyloidosis, plays an important role in the etiology of Alzheimer's disease. Thus, the investigation of transport, metabolism, and molecular interaction at the brain barrier systems has become a new focus in neurotoxicological research. This symposium will provide comprehensive reviews of the cutting edge research in this area, will discuss the use of new technologies in brain barrier studies, and will explore the brain barriers as the new frontier in drug development, toxicity evaluation, and neurodegenerative disorder research.

1609 BRAIN BARRIERS TOWARDS ORGANIC CHEMICALS: DRUG EFFLUX, BIOTRANSFORMATION AND MULTIDRUG RESISTANCE.

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The maintenance of brain homeostasis is achieved by 1) the specific properties of the blood-brain interfaces which control the exchange processes between the blood and the brain, i.e., the cerebral capillaries, the choroid plexuses (CPs), and the meningeal arachnoid; and 2) a unique cerebrospinal fluid (CSF) circulatory system in the central nervous system (CNS). These specific brain features protect the brain from exposure to different blood-borne drugs and toxins, and regulate the cerebral clearance of neurotoxicants. The tight junctions that seal both the endothelial cells of the parenchymal capillaries and the choroidal epithelial cells restrict the entry of polar compounds in the CNS. Brain efflux mediated by transporters such as the P-glycoprotein at the blood-brain barrier and members of the oatp(organic anion-tranporting polypeptide) and OAT(organic anion transport) families at the CP epithelium, also limits the cerebral bioavailability of various lipophilic, amphipathic or anionic compounds. Several drug metabolizing and antioxidant enzymes are present at both the blood-brain and blood-CSF barriers. In particular, CP appear to be a main site of detoxification in the brain. The use of an *in vitro* cellular model of this blood-CSF interface allows to demonstrate the existence of an effective metabolic barrier toward neurotoxins at the choroid plexus. This conceptual metabolic barrier is dependent upon the intracellular level of cofactors, such as glutathione, and results from active conjugation processes coupled to a polarized basolateral (i.e., blood-facing) efflux of the conjugated metabolites *via* a multidrug-resistance-

associated efflux pump. Reactive compounds, however, can also be generated at these interfaces by metabolic activation, a process that could lead to a disruption of brain homeostasis, should the antioxidant capacity of the interfaces be overflown.

1610 BLOOD-BRAIN BARRIER TRANSPORT OF METALS: IMPLICATIONS IN CNS HOMEOSTASIS OF ESSENTIAL AND NONESSENTIAL METALS.

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This lecture will highlight the transport of various metals (e.g. manganese and mercury) across the blood-brain barrier (BBB). It will emphasize the mechanisms of transport and address the means of both essential (manganese) and nonessential (mercury) metal transport by the BBB, *via* the mechanisms of ionic (iron) and molecular (methionine) mimicry, respectively. An important process in the toxicologic outcome of exposure to metals is their transport from plasma into the brain across the capillary endothelial cells that comprise the BBB. In order to cross this barrier, metal complexes must be either highly lipid soluble, or possess affinity for specific carrier-mediated transport systems within the endothelial cell plasma membrane. Little is known about manganese and mercury transport. The objectives of this lecture are, therefore, to identify the transport mechanism(s) of mercury and manganese, under the assumption that increased rate of metals transport across the BBB is reflected in elevated CNS concentrations and by inference increased neurotoxicity. Emphasis will be directed at experimental results derived from both *in vivo* and *in vitro* studies, as well as elaborating on the advantages and disadvantages of current methodologies for the assessment of transport kinetics of these metals, such as tissue culture techniques and *in vivo* microdialysis. (Supported by Public Health Service grant ES07331 and ES10563)

1611 INTERACTIONS BETWEEN TOXICANTS AND TIGHT BARRIERS.

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Tight barriers such as those formed by epithelial and brain endothelial cells prevent the passage of large molecules or those carrying charges from entering brain, thus providing protection for neural cells against xenobiotics. Nonetheless, toxicants are capable of passing through barriers. Some toxicants have been shown to increase nonspecific permeability by compromising the integrity of the blood brain barrier (BBB). A class of transporters that mediate uptake of metals, for example Pb, are the anion exchangers (AE). Because AE is highly expressed by choroid epithelium, it may explain why lead (Pb) is readily taken into the choroid plexus. However, physiological and anatomical studies have suggested that AE are unlikely involved in the transport of Pb through the intestinal barrier and the BBB. Instead, the major intestinal iron transporter, i.e., divalent metal transporter 1 (DMT1) which has been shown to transport a broad range of metals, may be involved. Overexpression of DMT1 in yeast by results in an increased uptake of Pb, whereas knockdown of DMT1 in intestinal epithelial cells results in a decreased uptake of Cd, suggesting that DMT1 mediates the transport of Pb and Cd. Interestingly, DMT1 requires H+ for its function and thus is not likely to be involved in the transport of Pb from the blood across the BBB. Pb at higher concentrations does not require a transporter because it induces a breakdown in the BBB. Organophosphate insecticides also increase permeability of toxicants by inducing a similar breakdown in the BBB. In summary, tight barriers express specific transporters for nutrients that can also transport toxic metals. Additionally, toxicants can increase permeability by compromising the integrity of the tight barriers.

1612 CONTRIBUTIONS OF BRAIN BARRIERS IN NEURODEGENERATIVE DISORDERS AND CHEMICAL-INDUCED NEUROTOXICITIES.

W. Zheng. *Environmental Health Sciences, Columbia University, New York, NY.*

Normal neurological function relies on the delicate chemical balance among neurons, neuroglia and their associated synaptic connections. The damaging effect of a chemical to the barriers can contribute substantially to chemical-induced neurotoxicity. Mechanistically, some chemicals directly disrupt barrier structure and increase its permeability, perturbing the chemical milieu and allowing for unregulated materials to come into contact with brain parenchyma. Other chemicals may not directly alter the morphology of the barriers, but rather impair barriers' regulatory function, bringing about neurotoxicity due to disordered cerebral homeostasis. Furthermore, certain chemicals prior to their entrance to the cerebral compartment are bio-activated or bio-deactivated by metabolizing enzymes present in brain barriers. Recent evidence also suggests that the structural and functional integrity of brain barriers is important in the etiology of Alzheimer's disease. The aging of the cerebral vascular structure, the ability of barriers to transport extracerebral beta-amyloid, and the production of beta-amyloid solubilizing macromolecules by the barriers, are amongst the possible contributive factors under considerable scrutiny, although the role of chemical exposure in disordered barriers of Alzheimer's remains poorly understood. In addition, the dysfunction in iron regulation at brain barriers caused by manganese (Mn) exposure has been suggested to underlie a Mn-induced Parkinsonism-like syndrome. This lecture examines the current understanding of the role of brain barriers in CNS disorders by focusing on chemical-induced neurotoxicity. The perspectives for future research needs will also be discussed.

1613 NIEHS SYMPOSIUM: ENVIRONMENTAL INFLUENCES ON CHILDHOOD ASTHMA.

K. M. Olden. *NIEHS, Research Triangle Park, NC.*

Childhood asthma is the subject of the symposium. This disease was chosen because of the strong link to environmental exposures, the gene-environment interactions, and because it represents an example of how basic research can be translated into intervention and prevention programs. In addition, childhood asthma is a chronic lung disorder of enormous public health importance that disproportionately affects minorities and persons of lower socioeconomic status. It is the most frequent cause of pediatric emergency room use and hospital admissions and is the leading cause of school absences. Despite improvements in diagnosis and management, and an increased understanding of the epidemiology, immunology, and biology of the disease, asthma prevalence, morbidity, and mortality have progressively increased over the past 15 years. To reduce the incidence of childhood asthma a multidisciplinary approach to the research effort is necessary. This symposium will include presentations that span research in epidemiology, molecular mechanisms, gene-environment interactions, environmental agents related to the disease, and intervention and prevention of childhood asthma.

1614 ENVIRONMENTAL INFLUENCES ON CHILDHOOD ASTHMA.

F. D. Martinez. *Arizona Respiratory Center, University of Arizona, Tucson, AZ.* Sponsor: K. Olden.

Significant advances have been made during the last ten years in our understanding of the environmental factors that may protect against or predispose to the development of asthma. The finding that most cases of asthma begin during the first years of life has focused the attention of many researchers to the factors that may influence the risk for asthma in these early years of development. The study of risk factors for asthma is complicated by the heterogeneous nature of the disease. Recurrent airway obstruction is the final common pathway for at least three different syndromes, which co-exist during infancy and childhood. "Transient infant wheezing" is confined to the first 2-3 years of life and is benign in course and mainly associated with maternal smoking during pregnancy. "Non-atopic wheezing" is a typical disease of infants and toddlers but may also persist up to the school years. It is characterized by attacks by wheezing mainly associated with wintertime infections. "Atopic asthma" is less frequent during the early years but becomes the most prevalent form of asthma by early adolescence. It is associated with chronic symptomatology, deficits in lung function, and persistence of symptoms into the adult years. It now appears that, whereas early microbial burden is a risk factor for transient infant wheezing and non-atopic asthma, several studies have shown that such burden is a protective factor against the development of atopic asthma. The mechanisms by which microbial burden in early life protects against the development of atopic asthma are beginning to be understood. Exposure to external "danger" signals that interact with the innate immune system may be necessary for the development of a mature, balanced immune response that may last for a lifetime. Lack of such microbial burden at crucial times at the development of the immune system may select certain forms of immune responsiveness that are associated with allergic sensitization, airway hyperresponsiveness, and chronic asthma systems in susceptible individuals.

1615 ENVIRONMENTAL AGENTS RELATED TO CHILDHOOD ASTHMA.

D. Zeldin. *NIEHS, Research Triangle Park, NC.* Sponsor: K. Olden.

Prior studies indicate that exposure to a variety of indoor allergens, such as those that derive from house dust mites and cockroaches, are important risk factors for the development of allergy and asthma in children. Moreover, recent studies indicate that bacterial endotoxin may be an important environmental factor in asthma

pathogenesis and that exposure to both allergens and endotoxin may cause a more severe inflammatory response than exposure to either stimulus alone. The National Allergen Survey, a descriptive study of allergen types/levels and endotoxin in floor and bedding dust in the nation's housing, is the first study to provide estimates of allergen and endotoxin exposure in the entire US population. The survey was conducted between 1998-1999 using a population-based, multi-stage area probability design which has yielded a sample that is representative of diverse geographic regions, socioeconomic groups and housing types. It encompasses 831 homes in 75 primary sampling units located throughout the country. The major endpoints are measurements of dust mite (Der f 1, Der p 1), cockroach (Bla g 1), cat (Fel d 1), dog (Can f 1), rodent (Mus m 1, Rat n 1) and fungal (Alternaria) allergen levels, and endotoxin in dust samples collected from an array of sites in the surveyed homes. This presentation will: (a) provide a brief overview of previous studies which demonstrate a relationship between indoor allergen/endotoxin exposure and asthma/allergy; (b) review preliminary data from the National Allergen Survey which provide current estimates of indoor allergen/endotoxin exposure in the US housing stock; and (c) discuss regional, socioeconomic, and housing factors that influence indoor allergen/endotoxin exposure.

1616 CELLULAR AND MOLECULAR MECHANISMS OF ASTHMA PATHOGENESIS.

M. Wills-Karp. *Division of Immunobiology, Childrens Hospital Medical Center, Cincinnati, OH.* Sponsor: D. Brown.

Asthma is characterized by variable airflow obstruction, airway hyperresponsiveness and airway inflammation. The inflammatory response in the asthmatic lung is characterized by infiltration of the airway wall with mast cells, lymphocytes and eosinophils. Although asthma is multifactorial in origin, recent advances suggest that asthma is an immune disease with a prominent role for T lymphocytes in the pathogenesis. As such, CD4+ T cells producing a Th2 pattern of cytokines (IL-4, IL-13, IL-5) have been hypothesized to play a pivotal role in the pathogenesis of this disease. The paradigmatic Th2 cytokines, IL-4 and IL-5, had been thought to be the primary regulators of the allergic response. However, recent studies from my group have shown that IL-13 is likely the central mediator of the effector phase of allergic responses. Specifically, we showed that blockade of IL-13 at the time of antigen challenge in antigen sensitized mice *via* administration of a sIL-13Rα2-Ig which only binds IL-13, ablated antigen induced AHR. Furthermore, delivery of the recombinant cytokine to the lungs of naive animals reproduced the entire allergic phenotype (i.e. AHR, eosinophilia, mucus hyperplasia). A role for IL-13 in human asthma has been suggested by the demonstration that IL-13 mRNA and protein levels are elevated in the lungs of asthmatics as compared to normal individuals. Although the exact mechanisms by which IL-13 induces AHR are currently not known, IL-13 has effects on a number of effector cells thought to be important in asthma including mast cells, B cells, eosinophils, airway epithelial cells, and smooth muscle cells. Grunig et al, 1998 have shown that T and B cells are not necessary for IL-13-induced AHR. Recent findings from my laboratory suggest that IL-5 and eosinophils are also not necessary for the effects of IL-13 on airway reactivity. As IL-13 receptors are expressed on airway smooth muscle cells, IL-13 may have direct effects on airway smooth muscle contractile function. Determination of the exact mechanisms by which IL-13 induces airway hyperresponsiveness awaits further studies.

1617 GENE-ENVIRONMENT INTERACTIONS IN CHILDHOOD ASTHMA.

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The prevalence of asthma morbidity and mortality has increased in industrialized countries at an alarmingly high rate for the last 20 years. The rates are disproportionately high in urban environments and in children, but the causes are unknown. It has become increasingly clear that genetic background is an important determinant. Inheritance patterns of asthma indicate that it is a complex quantitative disease. The complexity stems partly from the number of subphenotypes that characterize the disease, including airways hyperresponsiveness, inflammation, and allergic status. For each subphenotype, there may be a gene or set of genes that confer susceptibility or predisposition, and these genes interact to produce the asthma phenotype(s). Genome-wide searches for linkages of asthma subphenotypes have revealed a number of quantitative trait loci, each of which has potential candidate genes for the phenotype in question. Further, family and population studies have identified linkage of specific phenotypes with candidate genes. However, genetic background alone cannot account for the increasing asthma prevalence. The expression of the disease or its subphenotypes is likely dependent on environmental factors. That is, the relative risk of asthma for a specific gene or combination of genes may be a function of the frequency of exposure to the environmental agent, the strength of interaction between the genotype and the agent, and the specificity of the environmental effect with respect to the genotype. Potentially important environmental exposures for asthma include indoor air pollutants (NO2, tobacco smoke), outdoor air pollutants (ozone, PM2.5 and ultrafine, NO2, and SO2), and allergens such as house dust mite, cockroach, pollen, and cat dander. Assessment of exposure to these agents is critical to evaluating respective risk for each relative to specific genotypes. Understanding the interaction of environmental and genetic factors will lead to improved evaluation of risk of a specific genotype(s) for a given environmental exposure, and a potential means for intervention and prevention.

1618 A COMMUNITY-BASED PARTICIPATORY RESEARCH APPROACH TO REDUCING ENVIRONMENTAL TRIGGERS TO CHILDHOOD ASTHMA IN DETROIT.

B. A. Israel, E. A. Parker, T. Robbins, T. Keeler and T. Dvonch. *Health Behavior and Health Education, University of Michigan, Ann Arbor, MI.* Sponsor: K. Olden.

The Michigan Center for the Environment and Children's Health (MCECH), is one of the NIEHS/EPA, Centers for Children's Environmental Health and Disease Prevention. MCECH's research projects focus on: 1) determining the effects of allergen-induced local, excessive production of chemokines on the respiratory system; 2) assessing the separate and possible interaction effects of outdoor and indoor air quality on exacerbation of asthma in children; and 3) household and neighborhood level interventions focusing on reduction of environmental triggers for asthma among children in the southwest and east side of Detroit. The latter two projects have been combined to form Community Action Against Asthma (CAAA). A unique feature of the CAAA project is the use of a community-based participatory research approach. CAAA's household intervention employs Community Environmental Specialists (CES) who visit the homes of intervention participants using an individualized plan that has been developed jointly with the caregiver. This plan draws upon data such as, the results of the child's allergy skin-test, a household dust sample, the caregiver's responses on a baseline survey, and a household environmental assessment tool. Through this plan, the CES's are able to tailor the activities in their visit (e.g., education on asthma and indoor environmental triggers and social support); supplies (e.g., vacuum cleaners, mattress covers, cleaning supplies); integrated pest management services; and referrals to social service/health agencies to emphasize those changes likely to impact the child's asthma symptoms.

1619 IS THERE REPRODUCTIVE RISK ASSOCIATED WITH EXPOSURE TO DISINFECTION BY-PRODUCTS OF DRINKING WATER?

G. R. Klinefelter. *Reproductive Toxicology Division, USEPA, Research Triangle Park, NC.*

The Safe Drinking Water Act requires the US Environmental Protection Agency to establish national drinking water standards that protect the health of the 250 million people who get their drinking water from public water systems. Today there is considerable interest on the part of Stakeholders (i.e. Congress, water industry, environmental groups) in the potential adverse reproductive and developmental outcomes associated with exposure to chemical by-products formed during the process of drinking water disinfection. During summer 2002 the Federal Advisory Committee will cast its final decision on the existing Stage 2 DBP rule which sets the maximum contaminant level goal for by-products in finished drinking water. Toxicology data demonstrate that sensitive reproductive and developmental endpoints are perturbed by specific disinfection by-products (DBPs). Epidemiology data suggest an association between elevated DBPs and adverse reproductive outcomes such as spontaneous abortion, still birth, and developmental anomalies. While there are still insufficient epidemiology data to substantiate causality, the toxicologic data provide some degree of biological plausibility. Of particular interest are emerging toxicology data which: establish the potential for DBPs to adversely affect the developing reproductive system, indicate that animals with a longer period of reproductive development are more sensitive to DBP-induced effects, and identify novel biomarkers applicable to epidemiology. In addition, proteomic data provided from both developmental and reproductive studies might aid in the elucidation of the mode of action of the haloacids. However, both the toxicology as well as the epidemiology data suffer from weaknesses which make formal risk assessment difficult. Moreover, one of the most perplexing issues surrounding the risk assessment process of DBPs is the requisite cost-benefit analysis, and it is for this reason that Stakeholders are undergoing intensive debate.

1620 REPRODUCTIVE AND DEVELOPMENTAL EFFECTS ASSOCIATED WITH HUMAN EXPOSURES TO DISINFECTION BY-PRODUCTS.

J. S. Reif. *Department of Environmental Health, Colorado State University, Fort Collins, CO.* Sponsor: G. Klinefelter.

While chlorination, the primary method of disinfecting municipal water in North America, is pivotal to protect the public from water borne infectious agents, the numerous byproducts formed during disinfection (DBPs)are now considerd a poten-

tial health risk. The chemical mixture of DBPs include trihalomethanes, haloacetic acids, and haloacetonitriles/aldehydes. Reproductive endpoints are important potential outcomes of DBP exposure for several reasons: (1) due to the ubiquitous nature of the exposure, small increases in relative risk will have a significant public health impact; (2) the fetus and reproductive system are sensitive to environmental toxicants; and, (3) they can be effectively studied because the interval between exposure and outcome is short. The epidemiologic database evaluating this relationship remains sparse. To date, there have been 18 studies published; 9 relied on reported quarterly concentrations of THMs in water supplies serving the subjects' community as the exposure metric. The remainder used a qualitative exposure assessment to examine associations between water source or method of disinfection and risk of adverse reproductive outcome. Several studies found weak associations between exposure to DBPs and adverse reproductive outcomes including spontaneous abortion, stillbirth, low birth weight, intrauterine growth retardation, somatic parameters and developmental anomalies (e.g. heart, oral cleft and central nervous system malformations). Recent studies suggest that the brominated DBPs may be more hazardous. However, the findings are inconsistent and the epidemiologic evidence is insufficient to infer a causal relationship. Additional laboratory and human studies are needed before this issue can be resolved. Future human studies should incorporate improved methods of exposure assessment to reduce misclassification, focus on specific byproducts, especially the brominated species of the trihalomethanes and haloacetic acids, and include additional outcomes such as fertility, fecundity and potential effects on semen markers.

1621 DEVELOPMENTAL CONSEQUENCES OF EXPOSURE TO DISINFECTION BY-PRODUCTS IN ANIMAL MODELS.

E. S. Hunter. *Reproductive Toxicology Division, USEPA, Research Triangle Park, NC.* Sponsor: G. Klinefelter.

Disinfection by-products (DBPs) have been associated with a variety of adverse developmental outcomes in epidemiology studies including cardiac defects, neural tube defects, still births, and spontaneous abortions. In toxicology studies, the trihalomethanes (THMs) do not produce high rates of dysmorphogenesis, but selected THMs do cause pregnancy loss, i.e. full-litter resorptions (FLR) in rats. Serum luteinizing hormone (LH) is diminished when bromodichloromethane (BDCM) is administered to Fischer 344 rats during the LH-dependent period of pregnancy maintenance suggesting that perturbation of the endocrine axis may be responsible for FLR. The possibility that BDCM compromises LH responsivity is also being explored as this has more relevance to human pregnancy maintenance. Among the haloacetic acid (HA) class of DBPs, trichloroacetate (TCA) and dichloroacetate (DCA) each induce heart, eye and kidney defects in rats. Dibromoacetate (DBA) produces small/absent kidneys in mice, eye defects in rats and skeletal abnormalities in both species. Bromochloroacetate (BCA) exposure results in decreased fetal implants and live fetuses. In the rat, BCA reduced day 6 pup weights, and DBA induced pre-and postnatal loss and reduced pup weight on PND 1 and 6. When compared in mouse whole embryo culture (WEC) the relative potency of the HAs are BCA>DBA>DCA. Dose additivity of DCA, DBA and BCA was observed in rat WEC. Haloacetonitriles, another class of DBPs, induce FLR [dichloroacetonitrile {DCAN) and trichloroacetonitrile {TCAN)], decrease birth weight [chloroacetonitrile, DCAN, TCAN, bromochloroacetonitrile BCAN), dibromoacetonitrile (DBAN)], decreased postnatal weight gain (DCAN, BCAN, DBAN) and increased postnatal mortality (DCAN, TCAN). While most DBPs produce effects on development and neonatal growth, many are only effective at doses that produce maternal toxicity. *in vitro,* embryos show induction of cell death with little concurrent effect on the cell cycle. Mechanistic studies are focused on HA-induced alteration in signal transduction pathways.

1622 REPRODUCTIVE TOXICITY IN THE MALE RAT FOLLOWING EXPOSURES TO DISUBSTITUTED HALOACETIC ACIDS.

G. R. Klinefelter. *Reproductive Toxicology Division, USEPA, Research Triangle Park, NC.*

Over the past decade many studies have demonstrated that disubstituted haloacetic acids present in drinking water as a result of disinfection produce adverse reproductive consequences in the male rat. Equimolar exposure to these haloacids over 14 days alters spermatogenesis in a manner which would indicate alterations in sperm quality, and indeed, fertility of cauda epididymal sperm is significantly compromised. The reduction in fertility is associated with diminutions in proteins in detergent extracts of sperm. One protein, SP22, is highly correlated with resultant fertilty. Based on emerging data generated in humans using anti-SP22 antibodies, future drinking water epidemiology studies may incorporate an assessment this biomarker. It is interesting that when haloacid exposures span the duration of spermatogenesis (i.e. 70 days), Sertoli-cell only seminiferous tubules manifest, even 6 months after cessation of exposure. This exacerbation rather than recovery may be significant in species in which spermatogenesis is less efficient (e.g. humans). Evidence that the magnitude of effects increase with duration of exposure has also been provided by assessments of the developing reproductive system. It is now clear that the duration of exposure rather than the window of exposure accounts for significant delays in preputial separation. Again, implications are a concern for species with a longer period of reproductive develoment. Spermatogenesis, fertility (including mating ability), and puberty are each hormone dependent processes. That these processes are altered significantly by haloacids indicates endocrine disruption. Indeed, recent data suggest that these chemicals inhibit steroidogenesis. Attempts to identify the mode of action within the seminiferous tubule indicate several specific cytosolic proteins which are diminished by both *in vivo* and *in vitro* exposures. These proteins await identification. Elucidation of the time and concentration response of these proteins may help point the way to mode of haloacid-induced spermatogenic disruption.

1623 THE RABBIT MAY REPRESENT A BETTER ANIMAL MODEL FOR DETERMINING REPRODUCTIVE RISK ASSOCIATED WITH DBPS.

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Rabbits have a long quiescent period of reproductive development before puberty mimicking human development better than rodents. Moreover, longitudinal evaluations of endocrine profiles, sexual capacity, and semen quality are feasible in rabbits. These considerations suggest that the rabbit may be a more relevant animal model in evaluating human reproductive health risk. For the study of dibromoacetic acid (DBA), male and female rabbits were exposed to *via* drinking water from gestation day 15 through puberty (0, 1, 5, and 50 mg/kg). Six ejaculates (one every third day) were collected and sexual capacity and seminal quality were determined. Two untreated females were artificially inseminated with a known number of sperm from each experimental male and pregnancy outcome determined. Males in the DBA groups showed reduced sexual interest and ejaculation failures. Rabbits in the 50 mg group that completed ejaculation took more attempts (1.5 *vs.* 1 in control) and longer time (30 *vs.* 15 sec) to achieve ejaculation; two had no sperm in their ejaculates at least once. The conception rates for male rabbits in 0, 1, 5, and 50 mg groups were 85, 55, 65 and 55%, respectively. The individual conception rates of each of the treated groups were significantly lower than that of the control group. Male rats exposed similarly had reduced fertility at >50 mg/kg. Female rabbits in the DBA groups had reduced populations of primordial follicles; numbers were significantly lower in both the 5 and 50 mg/kg groups. Female mice exposed similarly to DBA, were found to have no differences in the populations of ovarian follicles. That sexual function, seminal quality (reflected by lowered fertility even at 1 mg/kg), and primordial follicle populations were more sensitive in rabbits compared to mice and rats, may reflect longer exposure during reproductive development in the rabbit. Thus, the rabbit may be a more useful model for human reproductive health risk assessment than rodents, particularly when cumulative risk is indicated and lowered low effects are anticipated.

1624 DO AVAILABLE TOXICOLOGY DATA ON DBPS IMPACT OUR ABILITY TO ASSESS HUMAN REPRODUCTIVE RISK ?

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The strengths of the recent animal data set are the sensitivity of the endpoints, the characterization of the DBP(s) employed, and the use of exposure during sensitive life stages. There is some concordance of outcomes from the *in vitro* to *in vivo* animal studies, and from *in vivo* animal studies to some epidemiologic studies. The weaknesses of the animal studies are often relevance of the route of administration, exposure levels, exposures to single DBPs rather than mixtures, risks of false positives/negatives (especially in *in vitro* work), and limited gestational exposure duration and endpoints. Epidemiology studies employ large populations and methods to correct for confounders, but there is limited, and nonconcurrent characterization of the DBPs (are surrogates such as TTHMs [total trihalomethanes] or HAAs [haloacetic acids] useful?) in the drinking water, varying and divergent results, bias, confounding, misclassification, and intrinsic variability in drinking water profiles and human populations. Formal risk assessment requires data which satisfy strict criteria (not yet met for the most part). Identified data gaps are being filled with improved developmental and reproductive toxicity studies in animals and mechanistically driven studies with well characterized, standardized drinking water, and more sophisticated evaluation of reproductive and developmental endpoints. Epidemiologic studies must concurrently better characterize the drinking water (at

the faucet), better ascertain reproductive outcomes, and control for confounders, etc. All of these studies are expensive, labor intensive, difficult, and of long duration. We must address the costs *versus* the human health benefits of such efforts. Is it reasonable to generate data which may/may not result in the reduction of MCLGs (maximum contaminant level goals) if cost savings resulting from possible human health benefit can not be determined ? In other words, does the benefit (if any) offset the cost both with respect to research and treatment plant MCLG reduction ? There is a hazard; is there sufficient risk?

1625 NON-CLINICAL TESTING OF PHARMACEUTICALS FOR PEDIATRIC USE.

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Since the issuance of the Pediatric Final Rule by the FDA in 1998, the importance of adequate testing for the safety and efficacy of pharmaceutical products in children has become clear. The Pediatric Rule defines what products require testing, when pediatric studies are needed (with waiver and referral provisions), and expected interactions with the FDA. The FDA Guidance for Industry for compliance with the Pediatric Rule is expected to provide further clarification of the expectations of the Rule. To support clinical studies of new pharmaceuticals in children, preclinical safety studies must be conducted. Many organ systems (eg., central nervous system, pulmonary, reproductive, immune, etc.) continue to develop postnatally, sometimes until adulthood, and develop at different rates in different species. Since standard reproductive and developmental toxicity studies as described by ICH guidelines do not cover direct exposure of offspring during the postnatal and peripubertal periods, special juvenile toxicity studies may be necessary. Depending upon the target organ system of particular concern, different periods of postnatal exposure, and different animal species, may be most appropriate. When designing juvenile toxicity studies, many practical considerations must be taken into account, such as feasibility of dosing, sampling procedures, and development of new methodologies for assessment of targets in young animals. Finally, the FDA's experiences in reviewing study designs and results of juvenile toxicity studies will provide insight into the future designs and conduct of these studies.

1626 UNDERSTANDING THE PEDIATRIC RULE: AN INDUSTRY PERSPECTIVE.

L. A. Dostal. *Drug Safety Evaluation, Pfizer Global Research & Development, Ann Arbor, MI.*

The Pediatric Rule was issued as a regulation effective April 1, 1999 and requires the sponsor of an NDA or marketed product to provide information on the efficacy and safety of the drug product in children. This presentation will describe the requirements of the Rule as it applies to new active ingredients or dosage forms that are used in a large number of pediatric patients, where there is a potential for significant improvement in treatment, or where there is no current therapy available for children. If the sponsor believes that none of these conditions apply, they may request a waiver of the studies, or a deferral of the pediatric assessment until after the NDA is submitted. Often the design of a pediatric plan is complicated by the development of a suitable pediatric formulation and issues of safety of dosing infants and children with new chemicals that are not already demonstrated to be safe and effective in adults. This presentation will cover various strategies and questions that could be considered in the design of an acceptable Pediatric Plan to address the Pediatric Rule.

1627 SPECIES DIFFERENCES IN POSTNATAL DEVELOPMENT OF TARGET ORGAN SYSTEMS.

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Animal studies have been used to investigate the response of immature systems for many years. Developmental toxicity studies have traditionally focused on the prenatal period of growth and maturation. Studies using juvenile animals are currently being designed to investigate developmental issues that occur postnatally in humans. Importantly, not all target organ systems develop during the same timeframe with reference to the time of birth for different species. Selecting the appropriate species and timing of dosing are critical to the success of postnatal developmental studies. A consideration in making this selection is the timeframe in which exposure is expected during the development and maturation of the given test species compared to humans. Differences in timing of growth and maturation between species can be considered using microscopic evaluation for cellular proliferation and differentiation, as in the case of postnatally developing alveoli, or by other methods appropriate for assessing organ functional capacity, as in the postnatally developing nervous system. In some cases, the immature systems of children can render the toxicity of a chemical to be lower than that occurring in the adult due to differences in metabolism. On the other hand, children may lack the ability to detoxify a chemical resulting in a higher level of toxicity when compared to adults. Organ systems that undergo major postnatal development or maturation include nervous, skeletal, renal, pulmonary, and reproductive. Examples of drugs that behave differently in adult (mature) and pediatric (immature) organ systems will be presented to illustrate the effects of differences between species and the timing of maturation in the study of postnatal development.

1628 PRACTICAL CONSIDERATIONS IN THE EXPERIMENTAL DESIGN OF JUVENILE ANIMAL STUDIES.

M. E. Hurtt. *Drug Safety Evaluation, Pfizer Global Research and Development, Groton, CT.*

The design of juvenile animal toxicology studies to support pediatric clinical trials is usually dependant on the identification of target organ toxicity in adult toxicology studies, the understanding of species differences in postnatal development of those target organs, as well as the clinical indication(s) and target population of the drug. The scientific question that the study is intended to address is a significant determinant of the design. Selecting the appropriate species is a critical component of the design of juvenile animal studies. In addition, a number of dose considerations are important including route, volume, selection, duration/period and vehicle. Other study design issues will be addressed such as need for pilot studies, pharmacokinetic/toxicokinetic considerations, animal handling, and identification and distribution of animals to dose groups. Specific examples of study designs will be presented to illustrate the importance of these issues in the design of juvenile animal studies.

1629 FDA EXPERIENCE WITH NONCLINICAL DATA SUBMITTED TO SUPPORT DRUG TRIALS IN PEDIATRIC PATIENTS.

K. L. Hastings. *Division of Special Pathogen and Immunologic Drug Products, Center for Drug Evaluation and Research, USFDA, Rockville, MD.* Sponsor: K. Hastings.

Pediatric patients may be more susceptible to certain drug toxicities compared to adults, possibly because organ systems could be more vulnerable to insult during development than when fully developed. There are a number of examples of increased susceptibility to drug toxicity in pediatric patients compared to adults. There are also examples of unique toxicities seen in this population that have not been observed in adults. The Center for Drug Evaluation and Research has received reports of nonclinical toxicology studies submitted to support the safety of clinical drug trials in infants and children: analysis of some of these data sets supports the idea that there can be adverse effects in pediatric patients which might not have been anticipated based on studies in relatively mature animals or adult humans. These observations indicate that nonclinical toxicology studies in immature animals might be valuable in determining the safety of clinical trials in pediatric subjects. Nonclinical toxicology studies have varied in design and the potential implications of these variations will be discussed with respect to pediatric risk issues.

1630 RECENT DIOXIN CONTAMINATION FROM AGENT ORANGE IN RESIDENTS OF A SOUTHERN VIETNAM CITY.

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Marked elevation of dioxin associated with the herbicide Agent Orange was recently found in most blood samples from Vietnamese living in Bien Hoa, a large city in southern Vietnam near Ho Chi Min City. This city is located near an airbase used for Agent Orange spray missions between 1962 and 1970. A spill of Agent Orange occurred at this airbase over 30 years prior to blood collection in 1999. Blood samples were collected, frozen and sent to a World Health Organization-certified dioxin laboratory for congener-specific analysis as part of a Vietnam Red Cross project. Dioxin congener that contaminated Agent Orange, 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), was elevated in the blood of 40 out of 42 (95%)

Bien Hoa residents. Median TCDD level was 67 parts per trillion (ppt) (Range 2.4 to 413 ppt). A comparison sample from over100 residents of Hanoi where Agent Orange was not used, had blood TCDD levels of 2 ppt. TCDD contamination was also found in some nearby soil and sediment samples. Persons new to this region and children born after Agent Orange spraying ended also had elevated TCDD levels. This TCDD uptake was recent and occurred decades after spraying ended. We hypothesize that a major route of current and past exposures is from the movement of dioxin from soil into river sediment, then into fish, and from fish consumption into people.

1631 SERUM TCDD LEVELS AND THYROID EFFECTS AMONG US AIR FORCE VETERANS.

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We assessed potential health effects of 2, 3, 7, 8-tetrachlorodibenzodioxin (TCDD) on thyroid function in US Air Force veterans involved in aerial spraying of herbicide Agent Orange during the Vietnam War from 1962 to 1971 (Operation Ranch Hand). We examined associations between serum TCDD level with levels of thyroxin (T4), thyroid stimulating hormone (TSH), triiodothyronin percent uptake (T3% uptake), free thyroxin index (FTI), as well as with incidence of thyroid diseases. Data were available for 1009 Ranch Hands and 1429 comparison veterans categorized into four exposure groups – Comparison, Background, Low and High. We found consistently elevated levels of TSH in the High exposure group of Ranch Hand over all five medical examinations. We also observed statistically significant dose-response relationship between TSH and TCDD levels in 1982, 1985, 1987, and 1992. The High group of Ranch Hands had also a higher risk of having abnormally high TSH levels over all five exams, with 1997 results approaching statistical significance. Overall, the incidence of thyroid diseases was not increased in Ranch Hand veterans. These findings suggest that in the highest exposure group of Ranch Hand veterans, TCDD may affect thyroid hormone metabolism and function.

1632 SEQUENCE VARIATION AND PHYLOGENETIC HISTORY OF THE *MURINE AH* LOCUS.

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The *Ah* locus encodes for the aryl hydrocarbon receptor (AHR) which plays an important toxicological and developmental role. Sequence variation at this locus was studied in 13 different mouse lines that included 8 laboratory strains, 2 *Mus musculus* subspecies, and 3 additional *Mus* species. The data presented represents the largest study of sequence variation across multiple mouse lines at a single locus (≈15.9 kb/mouse line). A total of 2, 213 polymorphisms were identified and used to estimate the polymorphism rates and characteristics at this locus. Among all mice, the average frequency of polymorphisms in the intronic regions was 20.3 SNPs/kb and the average exonic frequency was 14.1 SNPs/kb. Between laboratory strains, the average intronic and exonic frequencies dropped to 5.4 and 2.9 SNPs/kb, respectively. By comparison, polymorphism frequencies were significantly higher at this locus than those observed in whole genome scans. There were 111 non-synonymous polymorphisms that resulted in 42 different amino acid changes, of which only 10 amino acid changes have been previously identified. Based on the nucleotide sequence, the phylogenetic history of the gene showed mice from the Ah^{b2} and Ah^{d} alleles in separate branches while mice from the Ah^{b1} and Ah^{b3} alleles exhibited a more complex history. Evolutionarily, the AHR protein as a whole appears to be under purifying selective pressure (K_a:K_s ratio = 0.237). Despite significant functional constraint in the basic-helix-loop-helix and PAS domains, ligand binding is not constrained to the high affinity allele, which further supports the role of the AHR in development and its importance beyond the adaptive response to environmental toxicants.

1633 TCDD AND THE BIOLOGICAL CLOCK.

L. T. Frame[1], W. Li[1], D. Settachan[1], J. D. Miller[2], P. Patel[1] and R. L. Dickerson[1]. [1]*Pharmacology, Texas Tech Health Sciences Center, Lubbock, TX and* [2]*Cell and Neurobiology, Keck School of Medicine of USC, Los Angeles, CA.*

Sleep disturbances and other neurological disorders are associated with occupational exposure to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD, or dioxin). These problems may persist for years after exposure, significantly affecting quality of life. We hypothesize that TCDD causes permanent circadian rhythm disturbances, which can be modeled in rodents. Using an infrared beam detection system, we monitored deer mouse locomotor activity continuously in the dark for several weeks. We found that TCDD doses as low as 10 ng/kg (gavage, in corn oil) caused persistent phase-shifts of intrinsic locomotor rhythm. However, the timing of the dose and the concentration were critical. The magnitude of the phase-shift was greatest when the dose was administered by gavage (in corn oil) during the subjective night. Doses in the ng/kg range or less were associated with hyperactivity, while doses in the ug/kg range were associated with more severe pathological and biochemical changes, including weight loss, disheveled appearance, paw edema, and death after several weeks to months. Interestingly, this syndrome resembles rodent models of chronic sleep deprivation, as described in the scientific literature. Using immunohistochemistry and Western blot techniques, we characterized critical protein expression patterns within the suprachiasmatic nucleus (SCN) of the hypothalamus, where the master circadian pacemaker resides in mammals. Maximal diurnal expression of the clock-associated protein, mPER1, was delayed 6 hours by TCDD exposure during the dark cycle. Light and the endogenous pacemaker normally regulate the transcription of c-FOS, an immediate-early gene. However, with TCDD, expression of c-FOS protein was significantly increased in the SCN throughout the light/dark cycle. Entrainment of circadian rhythms to environmental chemicals represents a mechanism by which organisms may lose synchrony with their normal environment.

1634 EFFECTS OF TCDD ON RAT MOLAR DEVELOPMENT: THE CRITICAL TIME WINDOW.

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Dental defects, especially disturbances in tooth development, are sensitive endpoints of dioxin-induced toxicity in both humans and animals. In rats TCDD exposure results in dental defects ranging from pulpal perforation in incisors to complete block of third molar development. We determined the critical time window for the effect of TCDD on rat molar development in a TCDD-sensitive rat line. Dams were exposed to a single oral dose of 1 µg/kg TCDD on gestation day (GD) 11, 13 or 19, or on postnatal day (PND) 0, 2 or 4. In a cross-fostering study dams were exposed on GD15 and the pups from each exposed dam were transferred to a non-exposed dam and vice versa immediately after delivery. Pups were killed at the age of 40 days, the eruption status of the third molar observed by stereomicroscopy, and the presence of the molars confirmed by radiography. Highest frequency of missing molars was observed in pups exposed both *in utero* and *via* lactation, and only the development of third molars was blocked. Frequency of missing molars was the higher the earlier the dam was exposed: 64%, 50% and 26% of third molars were missing in offspring of dams exposed on GD11, 13 and 19, respectively. In the cross-fostering study, 13% of third molars were missing in pups exposed only *in utero* and no more than 1% of molars were missing in pups exposed only *via* lactation. TCDD exposure time-dependently retarded the eruption of third molars, whereas the eruption of incisors and opening of the eyes was accelerated. The results indicate that the critical time window of molar development comes to the end right after parturition. However, continued exposure during lactation may further amplify the response. It is possible that TCDD affects incisors and molars *via* different mechanisms. (Supported by the Academy of Finland, the Finnish Research Programme on Environmental Health, Project 42551, and EC Contract QLK4-CT-1999-01446)

1635 SUSCEPTIBILITY LOCI FOR PORPHYRIA AND LIVER INJURY IN THE INTERACTION OF TCDD WITH IRON IN MICE.

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Among the actions of the 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) is the induction of hepatic porphyria. This is similar to the most common disease of this type in humans, sporadic porphyria cutanea tarda (PCT). Evidence is consistent with the action of TCDD being mediated through binding to the aryl hydrocarbon receptor with different *Ahr* alleles in mouse strains apparently accounting for differential downstream gene expression and susceptibility. However, studies of TCDD-induced porphyria and liver injury evidence indicate that there must be interactions with other susceptibility genes, perhaps associated with iron metabolism. We performed a quantitative trait locus (QTL) analysis of an F_2 cross (204 individuals) between susceptible C57BL/6J (Ahr^{b-1}allele) and the highly resistant DBA/2 (Ahr^{d}allele) strains after treatment with TCDD and iron. Genome-wide scans of crosses were performed using polymerase chain reaction amplification of microsatellite DNA markers. To determine the location of susceptibility genes, the extreme phenotype cohorts for hepatic porphyria, plasma ALT and AST together with thymus size (48 each) were initially genotyped for 44 markers spread across the genome at an average of about 20 cM apart. After assessment by principle component analysis, all mice were genotyped with 6-9 markers on candidate chromosomes. For porphyria we found QTLs on chromosomes 11 and 14 in addition to

the *Ahr* gene (chromosome 12). SWR mice are syngenic for the *Ahr*^{b}allele with the DBA/2 strain but are susceptible to porphyria after elevation of hepatic iron. Analysis of SWRxD2 F_2 mice (242) treated with iron and TCDD showed a QTL on chromosome 11, as well as finding others on chromosomes 1 and 9, for both porphyria and plasma ALT as a measure of liver injury. These findings show, for the first time, the location of genes other than *Ahr* that modulate the hepatic porphyria and injury caused by TCDD in mice. Orthologous loci may contribute to the pathogenesis of human sporadic PCT.

1636 POSTNATAL DEVELOPMENT OF RESISTANCE TO ACUTE LETHALITY OF TCDD IN TWO TCDD-RESISTANT RAT STRAINS.

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The exceptional resistance to acute lethality of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) is related to a point mutation in the transactivation domain of the Ah receptor (AhR) in adult Han/Wistar (Kuopio) (H/W) rats. However, during fetal period H/W rats seem to be sensitive to at least teratogenic effects of TCDD. This study investigated the time window where resistance develops. HW rats, line A rats (a new line with the H/W-type mutated AhR), and line B rats (line with a normal AhR but moderately resistant due to an unidentified gene) were exposed to a single i.p. dose 2 - 56 days after birth (8 - 21 rats per group, occasionally 4 rats). H/W and line A rats received 1000 ug/kg; male and female B rats received 200 and 100 ug/kg, respectively. The LD50 doses of these strains/lines are >10000, >10000, 830, and 410 ug/kg, respectively. HW rats were sensitive (25 - 65 % died within 42 days) if the dose was given on postnatal days (PND) 5 - 9, but were resistant thereafter (0 - 33 % mortality). Line A rats were sensitive on PND 2 (47 - 57 % mortality) but resistant after PND 5 (0- 6 % mortality). Line B rats were very sensitive on PND 5 - 14 (all died) but developed resistance before PND 28 (no rats died if exposed after PND 28). The results show that H/W rats are sensitive to TCDD immediately after birth but develop the exceptional TCDD resistance to acute lethality within the first two weeks after birth. The moderate resistance of line B rats develops approximately at the time of weaning. It seems likely that within this time window there is a change in one or more AhR-mediated toxic pathways. Studies on this temporal change might bring forth new understanding on the mechanisms of action of AhR.

1637 *AHR(-/-)* AND *CYP1A2(-/-)* KNOCKOUT MICE ARE PROTECTED FROM TCDD-STIMULATED INCREASES IN REACTIVE OXYGEN FROM LIVER MITOCHONDRIA.

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TCDD (2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin) causes oxidative stress (OS) in liver and extrahepatic tissues. We have previously shown that TCDD increases mitochondrial respiration-dependent reactive oxygen (RO) production, an important component of TCDD-induced OS. We herein examined the dependence of mitochondrial RO on the aromatic hydrocarbon receptor (AHR) or cytochrome P450 1A2 (CYP1A2), proteins critical for TCDD-induced hepatotoxicity. Congenic female *Ahr(+/+)*, *Ahr(-/-)*, *Cyp1a2(+/+)*, and *Cyp1a2(-/-)* mice were treated with TCDD (15 µg/kg, i.p.) or corn oil vehicle on 3 consecutive days; liver mitochondria were examined 1 week following the first treatment. Whereas TCDD elevated succinate-stimulated mitochondrial H_2O_2 production in the *Ahr(+/+)* and *Cyp1a2(+/+)* mice, this increase did not occur in mitochondria from *Ahr(-/-)* or *Cyp1a2(-/-)* mice. Interestingly, while constitutive levels of H_2O_2 production from the *Ahr(-/-)* mitochondria were only 25% of that observed in *Ahr(+/+)* mitochondria, there was no difference between *Cyp1a2(+/+)* and *Cyp1a2(-/-)* mitochondria. Mitochondrial aconitase, an enzyme inactivated by superoxide, was decreased 44% by TCDD in the *Ahr(+/+)* and *Cyp1a2(+/+)* mice but not in the *Ahr(-/-)* or *Cyp1a2(-/-)* mice. TCDD treatment increased mitochondrial glutathione levels in the *Ahr(+/+)*, *Cyp1a2(+/+)* and *Cyp1a2(-/-)* mice, but not in the *Ahr(-/-) mice.* These results suggest that the AHR controls the expression of genes involved in the TCDD-induced mitochondrial RO production and that one of these genes is *Cyp1a2*. These results may be related to our observation, using Western immunoblot analysis, that shows TCDD causes the accumulation of CYP1A1 and CYP1A2 proteins in liver mitochondria. The mechanism by which CYP1 family members cause mitochondrial OS may be of fundamental importance to TCDD-induced toxicity. *Supported by NIH grants P30 ES06096, R01 ES10133, ES08147, and T32 ES07250.*

1638 TCDD-INDUCED GENE EXPRESSION PROFILE SUGGESTS THE INVOLVEMENT OF DEATH-RECEPTOR PATHWAY LEADING TO INDUCTION OF APOPTOSIS IN THE THYMUS.

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Previous studies from our laboratory demonstrated that TCDD triggers apoptosis in thymocytes. Recently, two major apoptotic pathways in mammalian cells have been described. In the first pathway known as the death-receptor pathway, members including Fas (CD95) and TNF receptor I are activated. The second, mitochondrial pathway is triggered primarily through the activation of pro-apoptotic members of the Bcl-2 family. In the current study, we used cDNA arrays to detect the apoptotic pathway initiated by TCDD. To this end, C57BL/6 mice were injected with 0.1-50 µg/kg body weight of TCDD and 1 or 3 days later, thymus and liver were analyzed for a wide range of apoptotic genes. In the thymus, 23 out of 37 genes involved in apoptosis were up-regulated by TCDD by a factor of 2 or more. In contrast, in the liver, only 16 out of 37 apoptotic genes were up-regulated. In the thymus, several genes belonging to the death- receptor pathway were up-regulated including FasL, caspase-8, casper, CRADD, RIP, DAXX, TNF, TRAF, Trail and TRIP following TCDD treatment. In contrast, the expression of anti-apoptotic members of Bcl-2 family including Bcl-xL and Bcl-2 and proapoptotic members such as Bad and Bax were not significantly up-regulated. In the spleen, 20 out of 22 apoptotic genes screened were up-regulated following TCDD treatment. The induction of caspases was seen at all doses of TCDD tested. Together, the current study suggests that TCDD-treatment induces the death-receptor rather than the mitochondrial pathway in the sensitive organs to induce apoptosis (Supported in part by grants from NIH: ES09098 and HL58641).

1639 ROLE OF AH RECEPTOR IN TCDD-INDUCED APOPTOSIS IN THE THYMUS.

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It is well established that 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent suppressor of the immune system. Exposure to TCDD has been associated with marked thymic atrophy. Despite extensive studies, the mechanistic aspects of TCDD immunotoxicity in the thymus are unclear. Many reports suggest that the immunotoxic effects of TCDD are regulated by the aryl hydrocarbon receptor (AhR). Previous studies from our laboratory have demonstrated that TCDD induces apoptosis in thymocytes. In the current study we tested the hypothesis that TCDD-induced apoptosis in thymocytes is regulated by the AhR. To test this hypothesis, alterations in the expression of various surface molecules in the thymus were measured as a predictable biomarker of apoptosis, as previously reported. To this end, C57BL/6 (Ah-high responder) and DBA/2 (Ah-low responder) mice were injected with 5 µg/kg TCDD (i.p.). Five days later, thymocytes were analyzed for phenotypic alterations using flow cytometry. At this dose, thymic atrophy was observed in TCDD-treated C57BL/6 but not DBA/2 mice when compared to the control mice. Consistent with our earlier reports, thymocytes from TCDD-treated C57BL/6 mice showed an increase in the density of expression of CD3 and CD44, and a decrease in the density of expression of CD4, CD8 and J11d markers, when compared to the vehicle-treated control group. These phenotypic alterations were associated with thymocytes undergoing apoptosis. Interestingly, thymocytes from TCDD-treated DBA/2 mice did not exhibit any phenotypic changes characteristic of apoptotic thymocytes. The thymocytes of C57BL/6 wild-type mice when examined using pathway-specific cDNA microarray showed marked up-regulation in several genes involved in apoptosis. In contrast, AhR knockout mice showed no significant up-regulation in apoptotic genes. Together, these data suggest that TCDD triggers apoptosis in the thymus by an AhR-dependent mechanism. (Supported in part by grants from NIH: ES09098, F31ES11562-01 and HL58641).

1640 COMPARATIVE GENE EXPRESSION RESPONSES IN RATS FOLLOWING ACUTE AND CHRONIC EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD).

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While humans are exposed to both persistent and non-persistent aromatic hydrocarbon receptor (AHR) ligands, it is believed that the sustained activation of the AHR response pathway by poorly metabolized ligands such as dioxins may be a key event in the development of associated adverse health effects such as cancer. The present study compares the *in vivo* response following acute or chronic exposure to

2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), a potent AHR ligand, in the livers of Sprague-Dawley rats. Animals were treated weekly by oral gavage with either: 700 ng TCDD/kg/week for 13 weeks, corn oil for 12 weeks followed by a single dose of 1ug TCDD/kg, or corn oil for 13 weeks; and killed 3 days after the final dose. Replicate pairwise two-color fluorescence cDNA microarray analyses were carried out using total RNA from each exposed group and pooled RNA from time-matched vehicle-treated controls. Initial analyses confirmed the induction of CYP1A1 and CYP1A2 after both chronic and acute exposure, and identified numerous known and unknown expressed sequence tags (ESTs) as being significantly altered, based on the observed fluorescence ratio distributions. To validate similar levels of AHR activation in the different exposure groups, levels of CYP1 RNAs were quantified in total RNA isolated from the livers of these animals using quantitative real-time RT-PCR. While expression of CYP1A1 and CYP1A2 was similar between both chronic and acute exposure, consistent with the expected similar liver levels of TCDD, expression of CYP1B1 RNA was significantly higher after chronic exposure compared with acute exposure. These data together indicate that chronic exposure can lead to differences in gene expression responses compared to single exposures and may provide further insights into comparative mechanisms of action/toxicity of persistent *versus* non-persistent AHR ligands.

1641 DYSREGULATION OF AHR, ARNT AND CYP1A1 CNS DEVELOPMENTAL EXPRESSION PROFILES AND EFFECTS ON BENZO(A)PYRENE METABOLISM IN F1 GENERATION PUPS FOLLOWING SUBACUTE INHALATION EXPOSURE OF TIMED PREGNANT DAMS.

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Very few studies have focused on mechanistically based dose-related neurotoxicity at the molecular level with respect to development of the central nervous system in F1 generation pups. To fill this important data gap and to highlight possible mechanistic pathways, a study was undertaken to determine the bioavailable dose as well as transcriptional changes of Ah Receptor (AhR), aryl hydrocarbon nuclear translocator (ARNT), and CYP1A1 associated with the transplacental disposition of Benzo(a)pyrene [B(a)P] metabolites in F1 generation pups. In this developmental range-finding study, laparotomy on GD 8 was performed on timed-pregnant rats followed by dosing *via* nose-only exposure for four hours a day for ten days (GD 11- GD 20) to three concentrations of a B(a)P : carbon black aerosol (0.025, 0.075 and 0.1 mg/m3). Bioavailability studies in F1 generaton pups revealed a dose-dependent increase in total B(a)P metabolites in brain tissue. Analysis of the qualitative distribution of metabolites in brain tissue indicated a predominance of the 7, 8; 9, 10-diols during the first postnatal week. The expression of the AhR, ARNT, and CYP1A1 was analyzed from the cerebrocortical, cerebellar, and hippocampal brain regions by using semiquantitative RT-PCR and Western Blotting to determine the temporal developmental expression profile from PND 0 through PND 30. The RT-PCR results, using β-actin as the internal standard demonstrate that inhaled B(a)P significantly downregulates AhR, and CYP1A1 in cerebrocortical, cerebellar and hippocampal tissue during the first postnatal week when the regulatory synergism between neuronalglial interactions is essential for programmed growth and differentiation. (Supported by U50NS41071, G12RRO3032, ES 00287, and GM 008037 to DBH).

1642 MIDGESTATIONAL, SUBACUTE, MIXED TCDD/ B(A)P EXPOSURE: EFFECTS ON F1 GENERATION PUPS IN RADIAL ARM MAZE PERFORMANCE AND TEMPORAL EXPRESSION OF DEVELOPMENTAL CNS NEONATAL MARKERS II.

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In order to test the hypothesis that mixed TCDD/B(a)P exposure to timed pregnant dams deleteriously affects cognition in F1 generation offspring, the following study was conducted. Laparotomy on GD 8 was performed on four groups of timed-pregnant rats including unexposed control, corn oil-dioxin, B(a)P: carbon black aerosol, and dioxin-B(a)P: carbon black aerosol. A dose of 700 ng/kg of dioxin was given by gavage on GD 14 to timed-pregnant dams followed by dosing *via* nose-only exposure for four hours a day for four days (GD 14 through 17) to 0.1 mg/m3 B(a)P : carbon black aerosol. On PND 40, three animals from each litter were tested on a working/reference memory task using an 8- arm, Radial Arm Maze (RAM). All arms were baited during shaping and testing. Compared to control males, the time required (latency) to locate a food pellet in the female mixed exposure group significantly increased by a factor of two by session 8. In contrast, male mixed exposure litter mate latency times were indistinguishable from control males after session 8. RT-RCR and western blot analysis from cerebrocortical extracts for AhR from PND 0 through PND 30 revealed a downregulation of AhR in the mixed exposure group as compared to controls. Brain nuclear extracts from F1 pups were analyzed by EMSA on PND 0 through PND 30. Premature Sp1-DNA binding was observed on PND 5 in the mixed exposure group as compared to the control group. The results suggest that mixed TCDD/B(a)P exposure causes: 1) modulation of the developmental expression profile of transcription factors involved in growth and differentiation in the developing CNS . A probable consequence of this modulation is to dysregulate the timetable of cellular development and growth in the CNS such that the phenotypic expression is 2) a significant reduction in spatial learning and memory. (Supported by U50NS41071, G12 RRO 3032, ES 00287, and GM 008037 to DBH).

1643 MIDGESTATIONAL, SUBACUTE, MIXED TCDD/ B(A)P EXPOSURE : EFFECTS ON F1 GENERATION LONG-TERM POTENTIATION AND TEMPORAL EXPRESSION OF DEVELOPMENTAL CNS NEONATAL MARKERS I.

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The purpose of this study was to access the effects of mixed TCDD/B(a)P exposure to timed pregnant dams on the cellular basis of learning and memory in F1 generation offspring. Laparotomy on GD 8 was performed on four groups of timed-pregnant rats including unexposed control, corn oil-dioxin, B(a)P: carbon black aerosol, and dioxin-B(a)P: carbon black aerosol. A dose of 700 ng/kg of dioxin was given by gavage on GD 14 to timed-pregnant dams followed by dosing *via* nose-only exposure for four hours a day for four days (GD 14 through 17) to 0.1 mg/m3 B(a)P : carbon black aerosol. A significant ($p<0.05$) reduction in the birth index was observed in the dioxin-B(a)P: carbon black aerosol exposed group. There were no significant differences relative to controls in liver and/or brain to body weight ratios. Electrophysiological studies in the dentate gyrus revealed a significant dimunition in I/O curves and in long term potentiation in the mixed exposure group relative to controls. RT-PCR developmental expression profiles for AhR as well as western blot analysis from PND 0 through PND 30 revealed downregulation of AhR in the mixed exposure group as compared to controls in cerebrocortical extracts. Brain nuclear extracts from F1 pups were analyzed by EMSA on PND 0 through PND 30. Premature Sp1-DNA binding was observed on PND 5 in the mixed exposure group as compared to the control group. In conclusion, the mixed TCDD/B(a)P exposure was shown to 1) modulate the developmental expression profile of transcription factors involved in growth and differentiation in the developing CNS and 2) cause a significant reduction in a persistent synaptic enhancement induced by high frequency stimulation of afferents which has been suggested to be an important component of the cellular basis of forms of learning and memory. (Supported by U50NS41071, G12 RRO 3032, ES 00287, and GM 008037 to DBH).

1644 MODULATION OF SELECTED APOPTOSIS-RELATED GENES DURING RETINOIC ACID-INDUCED DIFFERENTIATION IN MURINE EMBRYONIC STEM CELLS.

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Apoptosis plays an important role during embryonic development. Apoptotic cell death is executed by caspases and can be regulated by Bcl-2 family of genes. Selected apoptosis-related genes of the Bcl-2 family; pro-apoptotic Bax and Bad, anti-apoptotic Bcl-2 were investigated by ribonuclease protection assay (RPA) during differentiation of murine embryonic stem cells (ES), mediated by all-*trans*-retinoic acid (RA). The mRNA expression of caspase 3, caspase 6 and certain proinflammatory cytokines were also investigated simultaneously. The ES cells mimic *in vivo* differentiation and provide an excellent alternative method to study differentiation and apoptosis. The ES cells were grown on mitotically inactivated fibroblast feeder layers in Dulbecco's modified Eagle's medium supplemented with retinol free 15% Knock-Out Serum Replacement, leukemia inhibitory factor (LIF) and β-mercaptoethanol. Differentiation was initiated in the absence of feeder layers and LIF. Ribonuclease analysis revealed increased expression of Bax, Bad and no effect on Bcl-2 mRNA in RA-treated cells compared to the vehicle-treated cells. Increased mRNA expression of caspase 3 and caspase 6 in RA-exposed ES cells suggested that caspases played an important role in RA-mediated apoptosis during ES differentiation. Increased expression of TNFα and macrophage migration inhibitory factor (MIF) was noted in RA-treated cells on day 14 as compared to vehicle-treated cells. Increased expression of interferon gamma inducing factor (IGIF/IL-18) in RA-treated cells on day 14 and 17 did not translate to increased INFγ expression. Expression of other proinflammatory cytokines was detected at only at background levels. Results suggested that RA-mediated apoptosis during differentiation of ES cells involves caspase 3, caspase 6, Bad and Bax. Our data is consistent with the biochemical process of apoptosis since there is relative lack of other proinflammatory cytokines except TNFα, IGIF/IL-18 and MIF in RA-treated cells, whose function during ES differentiation is speculatory.

1645 MATERNAL IMMUNE STIMULATION REDUCES URETHANE-INDUCED CLEFT PALATE AND ALTERS MATERNAL SPLENIC AND FETAL PALATE GENE EXPRESSION.

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Maternal immune stimulation by diverse techniques, prior to conception or during gestation, has been shown to be effective in reducing chemical-induced birth defects in mice. Morphologic lesions that are reduced include cleft palate and associated craniofacial defects, digit and limb defects, tail malformations, and neural tube defect, caused by chemical agents TCDD, urethane, methylnitrosourea, cyclophosphamide, or valproic acid. Limited information is available regarding mechanisms by which such immune stimulation reduced fetal dysmorphogenesis. In the present studies, immune stimulation in urethane-treated pregnant mice increased splenic leukocyte number, including T helper and cytotoxic/suppressor cells, B cells and macrophages. An array consisting of 151 genes was used to study genes in these leukocytes that control cell cycle and apoptosis, cytokine/interleukin production, and signal transduction and transcription regulatory factors important in development. As might be expected, maternal immune stimulation up-regulated expression of leukocyte genes involved in cell proliferation and cytokine production. Interestingly, expression of genes in the fetal palate, including bcl2a, b, pkCa and p53 was restored to control levels by maternal immune stimulation. Possible relationships between maternal immune stimulation and altered maternal and fetal gene expression, and reduced urethane-induced cleft palate, remain to be investigated.

1646 EFFECTS OF PHORBOL 12-MYRISTATE 13-ACETATE (PMA) TREATMENT ON MORPHOLOGY AND GENE EXPRESSION OF *XENOPUS* EMBRYOS.

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Effects of PMA treatment on morphology and gene expression were studied using embryos of *Xenopus laevis* at stages 8 (blastula) and 15 (neurula). *Xenopus* embryos were obtained by induction of ovulation followed by *in vitro* fertilization. Embryos at stage 8 were obtained after incubation of the fertilized embryos at 23°C for 5 hours. PMA (100 ng/ml) or DMSO (0.01%, solvent used to dissolve PMA) was added to the embryos 2 hr after fertilization. Whereas embryos without PMA treatment progressed to blastula stage, PMA-treated embryos remained in a pre-blastula stage, suggesting that PMA treatment impaired differentiation. Embryos at stage 15 were obtained after incubation of the fertilized embryos at 18°C for 30 hours. PMA or DMSO was added to the embryos 21 hr after fertilization. Whereas embryos without PMA treatment showed typical morphology of neurula, PMA-treated embryos were in a pre-neurula stage. Embryos treated with 4 alpha-PMA, a stereoisomer of PMA that is ineffective at activating protein kinase C, showed typical neurual morphology. Embryos at stages 8 and 15 treated with PMA or DMSO were harvested and mRNA was isolated. The mRNA (3-5 µg) was reverse-transcribed with oligo$(dT)_{18\text{-}22}$ primer and dNTPs mixed with Cy3-dUTP (DMSO control) or Cy5-dUTP (PMA-treated). The Cy3- and Cy5-incorporated probes were mixed and microarray analyses were carried out using a glass chip printed with PCR products of *Xenopus* expressed sequence tag (EST) clones (duplicate of ~1, 100 genes). Microarray analyses of stage 8 and 15 embryos showed that PMA altered the gene expression profile differently in the two embryonic stages. These results suggested that, whereas cell differentiation was blocked after treatment of *Xenopus* embryos with PMA at both pre-blastula and pre-neurula stages, the PMA-responsive gene expression profiles were developmentally stage-specific. Sponsored by the NIEHS SBIR contract N43-ES-15462 & the NIEHS Center grant P30 ES06639.

1647 TEMPORAL CHANGES IN THE EXPRESSION OF TRANSCRIPTION FACTORS IN THE DEVELOPING LEAD-EXPOSED HIPPOCAMPUS AS DETERMINED BY MACROARRAY ANALYSIS.

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The exposure to lead (Pb) may alter developmental gene expression and brain development through selective effects on the transcriptional machinery. In this study macroarray analysis was used in an attempt to identify transcription factors that are involved in the response to Pb-exposure. Rat pups were exposed to Pb-acetate (0.2 %) from birth to weaning *via* lactating dams. On various postnatal days the hippocampi of control and lead-exposed pups were examined by macroarray analysis for changes in the developmental profiles of approximately 30 transcription factors, belonging to known transcription factor families. A number of transcription factors appear to be altered following lead exposure and have distinctive temporal patterns of expression. The most prominent changes were exhibited by the Sp and Oct families. Expression of Sp1 and Sp2 peaked on PND 5 and was diminished by PND 15 in Pb-exposed animals. By contrast, elevated Oct-1 & Oct-2 expression was sustained in these animals during the same period. These two transcription families appear to play a critical role in mediating Pb-induced disturbances in developmental gene expression. While the involvement of Sp transcription factors in the response to Pb exposure has been previously shown, the changes in Oct-1 and Oct-2 expression identify a novel target for Pb. Future work will focus on determining changes in Oct-1 and Oct-2 protein levels and DNA-binding and the role of the Oct family of transcriptions factors in the neurotoxic effects of Pb.

1648 2, 3, 7, 8-TETRACHORODIBENZO-*p*-DIOXIN (TCDD) REDUCES CARDIAC HYPOXIA AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXPRESSION IN THE CHICK EMBRYO.

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We have shown that TCDD inhibits coronary angiogenesis in the chick embryo. During normal embryo development cardiac hypoxia stimulates the expression of VEGF in the heart, which then stimulates coronary angiogenesis to occur. We tested the hypothesis that TCDD inhibits hypoxia-stimulated VEGF mRNA expression in the chick embryo heart. To estimate levels of cardiac hypoxia, we used a hypoxia-sensing drug, EF5, that is metabolically reduced by an oxygen-inhibitable nitroreductase. When tissues are hypoxic, more EF5 is reduced to a reactive intermediate which then binds to cellular macromolecules. Bound EF5 was visualized using a Cy-3-conjugated antibody. VEGF mRNA expression was assessed by *in situ* hybridization. To confirm EF5 binding and VEGF mRNA expression correlated with cardiac hypoxia, chick embryos on day 9 were incubated for 24 hr at 20% O_2 (normoxic) or 15% of O_2 (hypoxic). Hearts from the hypoxic embryos showed increased EF5 binding and VEGF mRNA expression, which localized primarily to the trabeculae, compared to hearts from normoxic embryos. To test the effect of TCDD on cardiac hypoxia and VEGF expression, fertile chicken eggs were injected with 5 ml corn oil (control) or 0.3 pmol TCDD/g in 5 ml corn oil prior to incubation. On day 10 semiquantitative analysis of EF5 staining showed a significantly decreased level of hypoxia in hearts from TCDD-treated embryos as compare to controls. Similarly, qualitative analysis showed that TCDD-treatment decreased VEGF mRNA expression in the cardiac trabeculae as compare to controls. We conclude that TCDD may inhibit coronary angiogenesis by down-regulating hypoxia-induced VEGF mRNA expression. Supported by NIH grant ES-09804.

1649 ANALYSIS OF GENE EXPRESSION PROFILES IN DIFFERENTIATING MURINE EMBRYONIC STEM CELLS.

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Pluripotent embryonic stem (ES) cells of the mouse are able to differentiate *in vitro* into a variety of different cell types. In the embryonic stem cell test (EST), an *in vitro* assay for the determination of the teratogenic potential of compounds, ES cells of the mouse (cell-line D3) are investigated for their preserved potency to differentiate into contracting cardiomyocytes following compound exposure. The inhibitory effect of a compound on differentiation is compared with its cytotoxicity on ES cells and on differentiated mouse 3T3 fibroblasts. To improve the EST, molecular endpoints of differentiation were examined for their potential to predict the embryotoxic potential of a compound. Gene expression of the ES cells was measured at different time points after initiation of differentiation using oligonucleotide microarrays (Affymetrix). The effect of the strongly embryotoxic compounds 5-fluorouracil and all-trans retinoic acid, the weakly embryotoxic diphenylhydantoin and dexamethasone as well as the non embryotoxic penicillin and ascorbic acid were investigated in comparison with untreated controls. Among the genes showing the strongest up-regulation in untreated cells between day 7 and day 10 of differentiation compared to undifferentiated ES cells, several genes coding for cardiac specific proteins could be found. This included atrial/fetal and ventricular myosin light chain 1, myosin light chain 2, alpha and beta cardiac myosin heavy chain and cardiac troponins. Also, up-regulation of further cell-type specific genes like hematopoietic, endothelial or neuronal genes could be seen. 5-fluorouracil and all-trans retinoic acid as well as diphenylhydantoin led to a down-regulation of the relative expression of cardiac specific genes, while dexamethasone induced an up-regulation of cardiac genes. Penicillin and ascorbic acid did not significantly affect the relative expression of cardiac genes. In conclusion, gene expression represents a effective endpoint to study compound induced changes in cardiac differentiation. The implementation in the EST will be evaluated.

1650 DEVELOPMENTAL EXPOSURE TO POLYCHLORINATED BIPHENYLS EXERTS THYROID HORMONE-LIKE EFFECTS ON THE EXPRESSION OF THYROID HORMONE-RESPONSIVE GENES IN THE FETAL RAT BRAIN.

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Background exposure to polychlorinated biphenyls (PCBs) during fetal development is associated with lower IQ and attention deficit in humans and clear neural defects in experimental animals. These effects may be caused by interfering with thyroid hormone signaling in as much as PCBs reduce serum thyroid hormone. However, the structure of some individual PCB congeners is reminiscent of that of thyroid hormone and some authors propose that PCBs may interact directly with the thyroid hormone receptor. Therefore, we initiated a study to determine whether maternal exposure to PCBs has an effect on specific thyroid hormone-responsive genes in the fetal rat brain, including RC3/Neurogranin, Neuroendocrine-Specific Protein A (NSP-A), and Oct-1. NSP-C, which is not affected by maternal thyroid status, was used as a negative control. Using *in situ* hybridization, we have found that PCBs increase the expression of RC3/Neurogranin and do not affect NSP-C expression. The remaining analyses are forthcoming. These studies are the first to address the effect of PCBs on thyroid hormone action in the fetal rat brain, an important question considering that all humans are contaminated with measurable levels of PCBs. Moreover, they justify a concerted effort to test whether individual PCB congeners can bind to the thyroid hormone receptor and transactivate gene expression. Supported by ES10026.

1651 EFFECTS OF SINGLE COMPONENTS (HYPERTHERMIA & BORIC ACID)AND MIXTURES ON MID-ORGANOGENESIS STAGE RAT EMBRYOS USING MICROARRAY ANALYSIS.

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The goal of this project is to examine the interaction of two developmental toxicants, hyperthermia and boric acid (BA), and their effects on the gene expression profile of mid-organogenesis rat embryos following maternal *in vivo* exposure. When administered singly on gestational day (GD) 10 in the rat, both agents cause similar developmental toxicity profiles (malformed/reduced numbers of thoracic vertebrae & ribs). When used in combination, the interaction of BA (oral dosing, 500 mg/kg) and hyperthermia (41C & 42C, rectal temp maintained for 5 min) was synergistic as manifested by an increased prevalence of sternal, thoracic, lumbar and sacral defects, and reduced numbers of presacral vertebrae. These effects seem to target newly developed somites or the presomitic mesoderm at the time of exposure. We have shown that adverse morphological, biochemical and molecular alterations occur as early as 1-3 hr post exposure. Here, we have used the microarray approach to map the gene expression profile in embryonic tissues containing the somitic region isolated 3 hrs after *in vivo* exposure to BA, 41 or 42C. Briefly, 33P labeled cDNA was synthesized from total embryonic RNA by reverse transcription. Labeled cDNAs were then hybridized to Atlas Rat Toxicology 1.2 arraysTM and exposures were recorded using an FX phosphorimager. The relative expression of each gene was determined using Atlas ImageTM software. Preliminary normalized data focusing on sets of genes belonging to DNA damage & repair, apoptosis, heat shock and cell signaling pathways indicate upregulated expression following 42C and even greater with BA+ 42C exposure but not 41C or BA+ 41C, suggestive of a synergistic mechanism of action in the high dose combination. Long term efforts are to build predictive models for risk assessment for single and combined exposures. Supported by a fellowship to WAH by ORISE. This abstract does not necessarily reflect EPA or FDA policies.

1652 MATERNAL THYROID HORMONE INCREASES THE EXPRESSION OF GENES THAT AFFECT GLIAL DETERMINATION IN THE EARLY CORTEX.

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Thyroid hormone (TH) is well known to play essential roles in brain development in both humans and animals. However, the specific developmental events affected by thyroid hormone are poorly understood. As a result, risk assessment is not based on specific end points of thyroid hormone action, often making the assignment of adverse effects difficult. To identify specific end points of TH action in the fetal brain, we have begun to examine members of the Notch signaling pathway, which transduces cell-cell contact information on decisions of cell fate during cortical development. The target genes of the pathway are the basic Helix-Loop-Helix (bHLH) genes Hairy Enhancer of Split homologues 1 and 5 (HES-1, HES-5), which encode transcriptional repressors thought to antagonize proneural bHLH genes and favor gliogenesis in the cortex. To test whether TH of maternal origin affects Notch signaling in the fetus, we treated pregnant rats (n=36) in one of 6 ways: 1) Untreated 2) propylthiouracil (PTU) + Saline 3) PTU + one of two doses of T4 24 hours prior to sacrifice 4) PTU + one of two doses of T4, 48 hours prior to sacrifice. The doses of T4 were calibrated to restore maternal blood concentrations to within the normal range, or to be 10-fold higher. Animals were sacrificed on gestational day 16 when the fetal cortex is actively producing both neurons and glia. Semi-quantitative *in situ* hybridization revealed that PTU decreased HES1 expression and T4 restored or increased HES 1 expression in a specific region within the fetal cortex. We conclude that thyroid hormone affects the Notch signaling pathway in the fetal cortex. Independently, we have found that TH does not affect neuronal proliferation of the early cortex; therefore, we are pursuing the hypothesis that TH increases the formation of glia at the expense of neurogenesis. Funded in part by ES10026.

1653 INTERSPECIES DIFFERENCES IN BBDR MODEL INPUT PARAMETERS AND THEIR IMPLICATION FOR MODELING TUMOR INCIDENCE.

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The utility of biologically-based Dose-Response (BBDR) models for predicting cancer risks in humans could be increased if we understood how model parameters scale across species and what these scaling relationships indicate about species-specific cancer risks. Yet parameter selection is often treated as trivial relative to statistical aspects of model formulation. We are identifying species-specific values from the literature for the initial cell number and rates of cell birth, death and mutation in both normal and initiated cells. Such data are difficult to obtain because they are rarely the focus of published studies or abstracts and must be gleaned from control data. Collection of cell cycle kinetic and cell number data for several tissues of interest has revealed varied patterns in cross-species scaling. For example, while hepatocyte number increases in a ratio of 1:3:850 among mice, rats and humans, thyroid follicular cell number increases in a ratio of 1:20:4500. Hepatocyte number scales linearly with organ weight but follicular cell number deviates from linearity due to structural differences in the rodent and human thyroid. Backround levels of cell proliferation and apoptosis are similar among the three species, with greater differences between tissues than between species. A review of spontaneous mutation frequencies indicates that, across all three species, mutation frequencies for all tissues except lymphocytes generally range from 1×10^{-5} to 8×10^{-5} per cell. Thus, most of the key parameters in a Moolgavkar-type carcinogenesis model appear to be similar across species. We are now developing a modeling approach in which these similarities in input parameters, species lifetime differences, and the background tumor incidence rates in rodent and human populations can be considered in a coherent manner. Other factors, such as different rates of DNA repair may also be included in the modeling. This work is supported by funding from the American Chemistry Council.

1654 APPLYING THE B6C3F1 MICE GROWTH MODEL USING WEIGHT DATA FROM A NTP BIOASSAY STUDY: ASSESSING DOSE-RESPONSE EFFECTS OF P-NITROBENZOIC ACID.

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Sponsor: D. Singh.

Biologically based Dose-Response (BBDR) models are being used increasingly by regulatory agencies for predicting biological risks in terms of the dose level of a particular chemical. The primary purpose of the dose-response model is to provide a descriptive summary of experimental data as function of dose. For BBDR models that are based on growth, empirical models establishing relationships between body weights and age in growing animals are needed. In this paper, we applied our newly developed B6C3F1 mice growth model in assessing the dose-response effects of p-nitrobenzoic acid on the growth curves and parameters of mice that were involved in a NTP bioassay study. Individual growth curves of these animals exposed to 0, 1250, 2500, or 5000 ppm of p-nitrobenzoic acid were fitted by the model using a nonlinear least squares technique. Our main objective was to determine if the chemical caused dose-dependent changes in the mean values of the model's parameters. Standard linear regression techniques were used to determine if the parameter

values varied with dose. The results showed that most of the parameters demonstrated a significant dose-response trend; some increased with dose, while others decreased. Some showed no significant changes, however. These results are preliminary, but important, for three primary reasons. First, they show evidence which suggests that low chronic levels of p-nitrobenzoic acid cause dose-dependent changes in the growth parameters of living mice. Second, although the model is purely empirical, it is a potentially useful tool for analyzing B6C3F1 mice weight growth data from NTP bioassay studies. Third, because the parameters of the model represent biological indices of growth, they can be used for BBDR modeling purposes. Disclaimer: The views expressed in this paper are those of the author (s) and do not necessarily reflect the views or policies of the USEPA.

1655 PROBABILISTIC FRAMEWORK FOR NON-CANCER RISK ASSESSMENTS.

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Non-cancer human health risk assessments use point values to develop risk estimates and thus impart a deterministic character to risk, which by definition is a probability phenomenon. The objective of the present work was to develop a probabilistic framework that eliminates the deficiencies of point values and to estimate non-cancer risk across the population without the use of uncertainty factors. Probability distribution functions (PDFs) were incorporated within a physiological modeling framework to account for the propagation of uncertainty/variability in exposure, kinetics and dynamics of volatile organic compounds. The integrated physiological-based toxicokinetic-toxicodynamic model for the dichloromethane(DCM)-induced carboxyhemoglobinemia in humans was used to illustrate the proposed probabilistic framework. The mechanistic parameters were represented by: (1) PDFs which provided both the value of the parameters and their frequency of occurrence and (2) the correlations among the parameters to characterize the inter-relationships among the parameters. Ambient exposure concentration was linked to dose absorbed by PDFs that described the variability of DCM exposure (indoors, outdoors, exposure frequency, exposure duration, exposure time) and population characteristics such as gender, age, and inhalation rates at different activity levels. The median and 95% confidence interval of carboxyhemoglobin (HBCO) in blood after a lifetime of continuous exposure were estimated. The distribution of risk (i.e., HBCO) in the population was thereby determined by taking into account the combined effects of the multiple sources of uncertainty/variability encountered in the exposure-tissue dose-tissue response continuum without the use of uncertainty factors. This approach provides an alternative to the default methodology. It shows that application of probability into non-cancer risk assessments can reduce uncertainties when establishing exposure limits for specific compounds and provide better assurance that established limits are adequately protective.

1656 A BIOLOGICALLY-BASED DYNAMIC MODEL FOR PREDICTING THE DISPOSITION OF FORMALDEHYDE AND ITS METABOLITES IN ANIMALS.

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A multi-compartment biologically-based dynamic model was developed to describe the time evolution of formaldehyde biodisposition in animals (rats, mice and rabbits) following different formaldehyde exposure scenarios. A model previously developed to describe the biodisposition of methanol and its metabolites in animals and humans (Bouchard et al., 2001) was used as a framework for the development of this model since methanol is metabolized mainly to formaldehyde in the animal and human body. In the model, a certain fraction of formaldehyde is readily oxidized to formate, a major fraction of which is rapidly converted to CO_2 and exhaled. The rest of formaldehyde is converted to unobserved by-products which are represented by a compartment with slow elimination. The dynamic of inter-compartment exchanges was described mathematically by a mass balance differential equation system. Parameters were estimated from metabolite time profiles published in the literature following either methanol or formaldehyde exposure. Data were available over longer periods of time after exposure to formaldehyde than was the case following methanol exposure. These data showed that time profiles of CO_2 exhalation in rats, mice and rabbits followed a bi-exponential pattern after formaldehyde exposure that was not apparent from previously published data on methanol exposure. The model reproduces these patterns by introducing a slow elimination route of unobserved formaldehyde by-products through metabolism to CO_2 and exhalation, in addition to the more rapid elimination from the formate body burden.

1657 REACTION NETWORK MODELING: A NEW MODELING APPROACH FOR BENZO(A)PYRENE BIOLOGICAL PATHWAYS.

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A new modeling approach, BioMOL (Biological application of Molecular Oriented Lumping), has been used to simulate the metabolic pathways of xenobiotics in a biological system. By setting the reaction rules, the reaction network is generated automatically at the molecular level. Graph theory is used to convert chemical structures and reaction rules into computer code. The atomic connectivity of the molecule is represented by the bond-electron matrix. The reaction matrix is a compact representation of bond formation and breakage caused by a chemical reaction. BENZO(A)PYRENE was used to build the first model by BioMOL since it is a human carcinogen as well as an ubiquitous environmental pollutant. Its metabolic pathway has been extensively studied and a great deal of information is available in the biomedical literature. The reaction rules used in this model include epoxidation of double bonds, hydrolyation of epoxide, NIH shift of arene oxide to phenol, oxidation of phenol to quinone, glucuronidation, sulfation, and glutathione conjugation. The rate constants are estimated based on the reaction family concept and Quantitative Structure Reactivity Correlations (QSRCs). The reactivity indexes, which are converted to rate constants by QSRCs, can be calculated by BioMOL-integrated semi-empirical quantum chemistry software. Candidate reactivity indexes include heat of reaction, π-electron density, and bond order. Key parameters were determined in a normal human epidermal keratinocyte cell culture system and used to verify the model built by BioMOL. This approach will be a promising tool for complex biological processes involved in chemical and chemical mixture toxicology. This work was supported by NIEHS Grant # R01 ES09655.

1658 NEW DEVELOPMENTS IN THREE-DIMENSIONAL MODELING OF THE RESPIRATORY TRACT.

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Advances in computer hardware, software and imaging technologies have enabled the rapid development of three-dimensional (3D) models of the lungs and nasal passages of the rat and human. Magnetic resonance (NMR) techniques were developed to image nasal and lung airways of rats at 50-μm resolution. The level of detail achieved by NMR (terminal bronchioles) is comparable to lung cast measurements but the geometry is obtained at far greater speed and accuracy. Semi-automated methods were used to translate imaging data from NMR, confocal microscopy and other formats (e.g. lung casts) into 3D mesh structures. Together, these approaches reduced the development time for initial 3D meshes, which will facilitate the processing of multiple rats. Subsequent mesh refinement time (artifact removal) is highly dependent upon the quality of the source imaging data. In addition to reconstructing airway surfaces, the models are being extended to include details captured by NMR of tissue structures and boundaries governing lung expansion/contraction (e.g. lobes, diaphragm, heart, etc). Confocal microscopic images of smooth muscles lining terminal airways of normal and asthmatic monkey lungs were also reconstructed into 3D meshes and merged with airway surface meshes. More robust airway structures will facilitate future modeling of particulate clearance, gas/vapor exchange or linkage with dynamic response models. The 3D models will form the basis for computational fluid dynamics modeling of inhalation, exhalation and deposition of particulate matter. The model can be manipulated to simulate the impact of altered lung structure or function to improve dosimetry predictions in normal *vs.* potentially sensitive individuals. Confirmation of the ability of the models to predict dosimetry at this level of detail will require new techniques for measuring airflows and particulate deposition in discrete regions of the respiratory tract.

1659 A PHYSIOLOGICALLY BASED PHARMACOKINETIC/PHARMACODYNAMIC MODEL FOR THE INSECTICIDE PARATHION IN THE RAT.

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A physiologically based pharmacokinetic/pharmacodynamic model was constructed for the insecticide parathion. Tissue/blood partition coefficients were determined by equilibrium dialysis, while metabolic constants were obtained from

previously published studies. Detoxification pathways included enzymatic pathways mediated by cytochromes P450 and A-esterase, as well as non-enzymatic pathways involving butyrylcholinesterase and carboxylesterase. The pharmacodynamic model describing the interaction of paraoxon with acetylcholinesterase included phosphorylation of the active site, as well as a peripheral binding site, that when occupied by paraoxon, reduced the appki for phosphorylation of the active site by paraoxon. The apparent Ki (appki) for paraoxon when the peripheral site was unoccupied, determined experimentally, was 0.90 $pM^{-1}h^{-1}$, while the same constant when the peripheral site was occupied by paraoxon was 0.00000125 $pM^{-1}h^{-1}$. Other significant events included in the pharmacodynamic model were reactivation of inhibited enzyme, aging, and normal synthesis and degradation of enzyme. Comparison of the simulation output with data from various publications involving different routes of administration of parathion and paraoxon in the rat, indicated that the model accurately simulated the pharmacokinetic disposition of parathion and paraoxon, and accurately simulated the degree of acetylcholinesterase inhibition in brain, diaphragm and blood, as well as butyrylcholinesterase and carboxylesterase inhibition in liver, blood, and heart/lungs following exposure to parathion or paraoxon.

1660 BIOLOGICALLY BASED MODELING OF CHLOROFORM CYTOLETHALITY AND REGENERATIVE CELLULAR PROLIFERATION.

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The cytolethality of chloroform occurs when the rate of generation of toxic metabolites exceeds the capacity of cellular protective mechanisms. In this study, a PBPK model for chloroform was extended to include cytolethality and regenerative cellular proliferation (RCP). This allowed calculation of a simulated labeling index (LI) that was compared with LI data from chloroform-exposed B6C3F1 mice and F344 rats. Key challenges in developing the model were (1) identifying the optimal form of the function linking chloroform metabolism to cytolethality and (2) defining a mechanism controlling the rate and extent of RCP. Possible forms of the linking function include (a) the rate of metabolism, (b) the AUC for the rate of metabolism over some interval, (c) the rate of metabolism ≥ a minimum rate, and (d) the AUC for the rate of metabolism ≥ a minimum rate over some interval. The actual biological mechanism of RCP is only partially understood, but involves growth factors and signaling pathways. In the interests of model parsimony, a simplified signaling submodel was developed. In this submodel, liver cells secrete a signal into blood and normal liver size is associated with a specific blood level of signal. As cytolethality reduces the number of cells, the level of signal falls. The difference between the control and exposed levels of signal is used as a driving force to increase the rate of cell division. This is an efficient means of simulating the LI data. Simulations with rate of metabolism and the AUC for rate of metabolism signaling submodels did not accurately describe the LI data, and the more complex submodels are being evaluated. This work will increase our understanding of the quantitative relationship between chloroform pharmacokinetics and cytolethality and support biologically based modeling of the dose-response for chloroform-induced cytolethality in humans.

1661 A COMPUTER DOCKING STUDY OF THE BINDING OF POLYCYCLIC AROMATIC HYDROCARBONS AND THEIR METABOLITES TO THE LIGAND BINDING DOMAIN OF THE ESTROGEN RECEPTOR.

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Polycyclic aromatic hydrocarbons (PAH) are a class of ubiquitous, anthropogenic chemicals found in the environment. In the present study, we use computational methods to evaluate their potential estrogenicity and the contribution chemicals in this class make to environmental estrogenicity. Classical docking methods, Dock and Affinity, are used to evaluate the potential binding affinity of chemicals in this class and their metabolites to the published crystal structures of the ligand binding domain of the estrogen receptor. These methods, with a molecular mechanics interaction energy scoring function, were able to place estradiol within an RMSD of 0.4 Å from its binding position determined by x-ray crystallography. The scores obtained in this manner show wide variation for the PAHs and their metabolites. They depend on PAH type and the three dimensional structure of the metabolites. For example, (-)-anti-benzo[c]phenanthrene diolepoxide is a much better binder in this model than the (+) enantiomer. A few dominant modes of binding have been identified and will be presented. These results will be compared to the results for known binders. Semi-empirical quantum mechanical methods were also used to compute the interaction energy of the most stable structures obtained from the classical computer docking experiments. These quantum mechanical calculations provide a quantitative description of the interaction between the ligand and the receptor and contain elements that are omitted from the classical scoring function. The comparison of these results demonstrates the importance of the nonclassical interaction terms for molecules that have pi electron systems. (This work does not necessarily reflect EPA policy. K. W. Brown was funded by EPA/UNC Toxicology Research Program Training Agreement CT902908 and CT827206.)

1662 THE EFFECTS OF ATRAZINE ON THE ABILITY OF ESTRADIOL TO BIND TO ESTROGEN RECEPTOR IN THE HYPOTHALAMUS.

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Atrazine (ATRA), a chloro-triazine herbicide, is widely applied throughout the United States. At high doses, it suppresses the preovulatory luteinizing hormone (LH) surge in female rats. Although the site of action is the hypothalamus, the mechanism is unknown. Estradiol (E2) acts on hypothalamic neurons to initiate the LH surge. Chloro-triazines have weak anti-estrogenic effects in some assays. Our studies determined if ATRA's anti - estrogenic properties are responsible for the diminished LH surge in female rats. We verified this by an *in vitro* ER competition binding study with ATRA and 3H-E2. ATRA (0-750uM) caused a dose-dependent decrease in E2 binding to ER, independent of pre-incubation. Decreased responsiveness of the hypothalamus to E2, caused by ATRA, could interfere with the E2 - mediated positive feedback required for the LH surge. To test this, the following *in vivo* studies were performed: (1) we confirmed the ability of ATRA to blunt the LH surge in ovariectomized, E2 - primed female rats. At 300 mg/kg/day for 5 days, it significantly suppressed the surge. The remaining studies used this treatment regimen. (2) Using in - situ hybridization, we assessed changes in progesterone receptor (PR) mRNA expression following *in vivo* exposure to ATRA. PR mRNA is upregulated in response to activation of ER - E2 complex in the nucleus. If ATRA decreased ability to bind ER, PR mRNA would not be increased. ATRA treatment caused no change in PR mRNA expression in various hypothalamic regions compared to control. (3) An *in vivo* ER nuclear exchange assay determined changes of occupied hypothalamic ER after ATRA and estrogen treatment. ATRA had no effect on the ability of ER to bind E2. Therefore, despite evidence of anti-estrogenicity, this property of ATRA is unlikely to be involved in suppression of the LH surge. This research was funded by Superfund Grant (5 P42 ES05949).

1662A A SHORT-TERM DOSING MODEL FOR DETECTING THE EFFECTS OF ENVIRONMENTAL CONTAMINANTS ON THYROID HORMONES IN THE RAT: EFFECTS OF PESTICIDES.

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Recently, a short-term rat dosing model has been developed to examine the effects of environmental mixtures on thyroid homeostasis (TH). Prototypic chemicals such as dioxins, polychlorinated biphenyls and polybrominated diphenyl ethers have been tested and shown to adversely impact both neurological development and TH, primarily by up regulating catabolism of thyroid hormones by liver enzymes. Current efforts examined the effects of select pesticides in this model. Female Long Evans rats (28 days old) were orally dosed for four consecutive days with the dithiocarbamate fungicide mancozeb (MAN) or the herbicide pronamide (PRO) (0, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, 500, 1000 mg/kg/day), or the dithiocarbamate fungicide thiram (THI) (0, 6.25, 12.5, 25, 50, 100, 200, 400, 800 mg/kg/day). Serum and liver samples were collected 24 hrs after the last dose. Triiodothyronine (T3) and thyroxine (T4) were measured *via* radioimmunoassays. Hepatic ethoxy-resorufin-O-deethylase (EROD), pentoxy-resorufin-O-deethylase (PROD), and uridinediphosphate-glucuronsyltransferase (UDPGT), were determined in hepatic microsomal fractions. Liver-to-body weight ratios (LBR) increased for PRO at doses >125 mg/kg/day. MAN at >500 mg/kg/day, and THI at >400 mg/kg/day. PRO, MAN and THI all produced similar dose-related decreases in T4, with estimated ED50s of approximately 250 mg/kg/day. Potencies for decreases in T3 were THI > PRO > MAN. Maximal suppression of T3 and T4 was ~50% and 80% at the highest doses, respectively. Neither MAN or THI caused changes in EROD or PROD. These data suggest that this rodent dosing model is sensitive to short-term pertubations in thyroid hormones caused by these pesticides. This abstract does not necessarily reflect the policy of the USEPA.

1663 17β-ESTRADIOL- AND 4-HYDROXYTAMOXIFEN-INDUCED TRANSACTIVATION IN BREAST AND ENDOMETRIAL CANCER CELLS IS DEPENDENT ON ER-SUBTYPE, CELL AND PROMOTER CONTEXT.

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The pattern of transcriptional activation by 17β-estradiol (E2) and 4-hydroxytamoxifen (4-OHT) was determined in ZR-75 and MDA-MB-231 breast, ECC1 and HEC1A endometrial and HepG2 liver cancer cell lines cotransfected with E2-responsive constructs and wild-type estrogen receptor α (ER-WT) or ERβ (ERβ-WT) or variant forms of ERα expressing activation function 1 (AF1; ER-AF1) or AF2 (ER-AF2). The E2-responsive constructs contained promoter inserts from the human complement C3 (pC3-luc), human cathepsin D (pCD-CAT) and rat creatine kinase B (pCKB-CAT) genes. Minimal ERβ-dependent transactivation (<2.5-fold induction) was observed for E2 only in ECC1 and MDA-MB-231 cells transfected with pCKB-CAT or pC3-luc, whereas 4-OHT was inactive as an ERβ agonist for all promoters in the four cell lines. The pattern of E2- or 4-OHT-activated gene expression and their interactions were highly complex and dependent on cell and promoter context and form of cotransfected ERα. For example, E2 did not activate pCD in HepG2 cells transfected with wild-type or variant ERα, whereas E2 activated reporter gene activity in the four endometrial/breast cancer cell lines transfected with ER-WT and pCD, pCKB or pC3. However, hormone activation of these constructs by ER-AF1 or ER-AF2 was highly variable among the different cell lines and even in the same cell line transfected with the three E2-responsive constructs. Similar variability was observed for 4-OHT suggesting that transcriptional activation by E2 and 4-OHT induces recruitment of different transcription factor complexes that are dependent not only on the cell type but also the gene promoter. (Supported by NIH ES04917 and ES09106)

1664 EVALUATION OF POTENTIAL ESTROGENIC PROPERTIES OF OCTAMETHYLCYCLOTETRASILOXANE (D4) USING THE MCF-7 CELL LINE.

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The objective of this research was to evaluate the potential estrogenic properties of octamethylcyclotetrasiloxane (D4). Using an *in vitro* estrogen responsive reporter gene system followed by Northern blot analysis, D4, estradiol (E2), and diethylstilbestrol were evaluated using the MCF-7 human cell line. Treatment with D4 for 24 hours at an applied dose of 0.1 - 10μM resulted in a dose-dependent increase in pS2 expression. As expected, all of the positive control compounds resulted in dose-dependent increase in mRNA expression. Linear regression analyses indicated that D4 was 74, 009 times less potent than E2 with the compounds showing equivalent efficacy at the highest dose. Maximal mRNA expression was seen between 6 and 24 hours, although exposure at shorter times resulted in increasing levels of mRNA. The loss of D4 in the medium was monitored and there was a rapid loss of compound, which reached a plateau around 12 hours with less than 10% remaining. The potential for D4 to act as an anti-estrogen was evaluated comparing the E2 Dose-Response curve with and without co-incubation with 1.0 μM D4. This regression analysis resulted in parallel slopes with statistically indistinguishable y-intercepts, indicating that the D4 is not reducing the potency of E2 under these conditions. Preliminary receptor binding studies were carried out to examine the binding of D4 to ER-α and ER-β. Studies indicate that the D4 can weakly compete for the estrogen receptor, both ER-α and ER-β. D4 did not reduce E2 binding beyond 70% of the control value even at D4 concentrations of 250μM. This data suggests that D4 can elicit an estrogenic effect that is dose-dependent with no significant anti-estrogenic activity. Binding data indicate that the effect is mediated through the estrogen receptor. Supported in part by Silicones Environmental, Health and Safety Council of North America.

1665 CELL CONTEXT-DEPENDENT DIFFERENCES IN THE MECHANISM OF ESTROGEN REGULATION OF E2F1 IN BREAST CANCER CELLS.

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E2F1 is a member of the E2F family of transcription factors which plays an important role in cell cycle progression and cell proliferation. E2F1 is induced by 17β-estradiol (E2) in breast cancer cells and contributes to hormone-induced proliferation of estrogen receptor (ER)-positive cell lines. Mutation and deletion analysis of the E2-responsive -146 to -54 or -169 to -54 regions of the E2F1 gene promoter using transient transfection assays demonstrates that hormone-induced transactivation in MCF-7 cells requires at least one of three GC-rich sites (-169 to -111) that bind ERα/Sp1 and cooperatively interacts with two downstream CCAAT motifs (-122 to -54) that bind NFYA. Transient transfection of E2-responsive constructs pE2F1a (-169 to -54) or pE2F1b (-146 to -54) containing E2F1 gene promoter inserts are induced ≥ 3.5-fold in both ER-positive MCF-7 and ZR-75 cells. Gel mobility assays of nuclear extracts from both cell lines gave comparable patterns of retarded bands and confirm that Sp and NFY proteins bind upstream (^{32}P-169/-111) and downstream (^{32}P-122/-54) oligonucleotides, respectively. Deletion analysis of the E2F1 promoter in ZR-75 cells using pE2F1c and pE2F1d (containing -169 to -111 and -122 to -54 E2F1 promoter inserts) showed that E2 induced a 2.5- and 3.5-fold increase in reporter gene activity, whereas these constructs were inactive in MCF-7 cells. These results demonstrate that E2F1 is differentially regulated by cooperative ERα/Sp1-NFYA interactions in MCF-7 cells and by independent ERα/Sp1 and ERα activation of CCAAT binding proteins in ZR-75 cells. Current studies are investigating the novel genomic/non-genomic pathways associated with hormonal activation through CCAAT motifs. (Supported by NIH ES04176 and ES09106)

1666 ESTROGENIC RESPONSES TO GENISTEIN IN JAPANESE MEDAKA (*ORYZIAS LATIPES*).

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Although the phytoestrogen genistein has been determined to be weakly estrogenic, the change in endocrine function associated with genistein exposure is not necessarily expected to mirror the physiological changes following exposure to endogenous estrogens. Because of the potential for genistein to compete with 17-β-estradiol at the receptor, some estrogenic responses might be diminished in the presence of genistein. Thus, this study was designed to determine the estrogenic effect of the phytoestrogen genistein on endocrine physiology in adult Japanese medaka (*Oryzias latipes*) relative to 17-β-estradiol. Adult animals of both sexes were exposed to 75ng/fish, 750ng/fish, and 30μg/fish (average fish weight was 0.26g/fish) of genistein by i. p. injection. A positive control group was similarly treated with 300ng/fish of 17-β-estradiol, while a negative control group received a vehicle only (corn oil) injection. Several assays were used to determine the extent and type of changes in the endocrine system of treated animals. Vitellogenin, while induced by estradiol, was not increased in the liver of individuals treated with genistein. Genistein treatment at higher doses increased the estradiol production by ovaries more than estradiol treatment. In males, genistein treatment resulted in decreased testosterone production from *ex vivo* testis and a comparable reduction in circulating testosterone level. The changes in endocrine physiology in medaka in response to genistein are similar to that of estradiol. However, some endpoints are more sensitive to estradiol treatment (vitellogenin) while others are more sensitive to genistein (male testosterone and female ovarian estrogenesis). Future studies with dietary supplements will build on these results, which will serve as a basis for testing individual components of plant extracts for estrogenic activity.

1667 DIFFERENTIAL EXPRESSION OF THREE ESTROGEN RECEPTOR ISOYPES DURING LARGEMOUTH BASS *(MICROPTERUS SALMOIDES)* REPRODUCTION AND FOLLOWING EXPOSURE TO NONYLPHENOL.

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Discovery of multiple estrogen receptor (ER) isotypes raises questions regarding their role in gene regulation by endogenous estradiol and xenoestrogens. Here we report the discovery of three ER isotypes in largemouth bass (LMB), ERα, ERβ, and ERγ. Using real-time quantitative PCR we have determined that these isotypes are differentially expressed in brain, pituitary, gonad, and liver tissues in vitellogenic females. For example, ERα and ERβ are more highly expressed than ERγ in the liver. However, ERγ expression is greater than both of the other ER isotypes in gonadal tissue. This differential tissue distribution pattern suggests they play very different roles during the reproductive process. To begin to understand their roles in reproduction we measured the expression pattern of each ER in adult female LMB during normal oocyte development. Liver tissue was collected at approximately two-week intervals from October through June. Expression of each ER and vitellogenin (VTG) mRNA were quantitated using real-time PCR. We also measured plasma VTG protein levels by direct ELISA. Results show that each ER isotype displays a distinct pattern of expression. There is an apparent correlation between ERα and VTG mRNA and VTG protein levels. ERβ however, appears to be down regulated during peak vitellogenesis. ERγ expression remain fairly constant throughout reproduction suggesting it plays a minimal role in gene regulation in the liver. To

determine if nonylphenol (NP) treatment induced similar expression profiles of the ERs we injected adult male LMB with 50 mg/kg or 500 mg/kg, and sampled tissues after 48 hours. Both NP treatments increased the production of plasma VTG and ERα mRNA in a dose dependent manner. The results from the NP injection are similar to the results of the seasonal study, and both suggest that ERα plays a greater role in the regulation of VTG.

1668 THE ESTROGENIC PROPERTIES OF 3, 3'-DIINDOLYLMETHANE (DIM) ARE DEPENDENT UPON CYTOCHROME P450 (CYP) METABOLISM.

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Indole-3-carbinol (I3C) is a glucosinolate hydrolysis product from cruciferous vegetables that has been found to have chemoprotective effects in several animal models. However, I3C given long-term postinitation promotes liver carcinogenesis in trout and rat models, but inhibits hepatic tumors in mice similar to the actions of 17β-estradiol (E2). A major acid condensation product of I3C, DIM, is estrogenic in precision trout liver slices and accounts for the majority of the estrogenicity of I3C in trout *in vivo*. This evidence, along with the species selectivity of I3C as a hepatic tumor modulator, suggests an estrogenic mechanism of action for hepatocarcinogenesis. In this study, we demonstrate that DIM requires CYP-dependent metabolism to the active estrogen. Trout liver slices were incubated with 0.2% DMSO, 100 nM E2 or 20 μM DIM. CYP metabolism was inhibited in these slices with 20 μM SKF 525-A (SKF) or ketoconazole (KC). Vitellogenin (VTG) induction in E2-treated slices was not affected by either CYP inhibitor whereas VTG induction by DIM was significantly decreased 79% and 86% in slices treated with KC and SKF, respectively ($p < 0.05$). These data further support the importance of estrogenic mechanisms in tumor modulation by I3C and suggest that species differences in hepatic tumor promotion are due to the CYP-specific metabolism of DIM to estrogenic metabolites. Supported by PHS grants ES03850 and ES07060 from the NIH.

1669 THE REGULATION OF THE STEROIDOGENIC ACUTE REGULATORY PROTEIN (STAR PROTEIN) IN LARGEMOUTH BASS (MICROPTERUS SALMOIDES).

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The Steroidogenic Acute Regulatory Protein (StAR) transports cholesterol across the mitochondrial membrane, making cholesterol available to be metabolized into steroid hormones. Some environmental contaminants have been found to alter the levels of steroid hormones, which could occur through targeting the expression of the StAR Protein. We obtained gonad samples of largemouth bass (LMB) at two week intervals throughout a year to determine the normal mRNA levels of the StAR Protein and it's correlation with steroid hormone levels. Total RNA was isolated from gonads and mRNA levels of StAR were then quantitated using real-time PCR with Taqman technology. Primers for the real-time PCR assay were designed from a partial, 345 base pair sequence of the LMB StAR Protein previously cloned in our Lab.. The levels of StAR in LMB go from an average of 0.2 pg StAR mRNA/ug of total RNA during the quiescent period in the fall to a peak of 7.3 pg StAR mRNA/ug of RNA during the primary reproductive season. The expression pattern of StAR correlates with the expression pattern of 17-beta-estradiol, as would be expected since it is the rate limiting step in steroid synthesis. Establishing the normal levels of StAR in LMB during the reproductive season will set baseline parameters for studying the effects of environmental contaminants on StAR expression. It has been shown already that several contaminants, including herbicides and insecticides, decrease the expression of the StAR Protein in mammalian cell lines (Walsh et al, 2000, Environ Health Perspectives, 108(8):769). Fish exposed to contaminants from paper mills have been extensively shown to exhibit decreased steroid levels, possibly through alteration of StAR expression. LMB is an excellent model for studying the effects of contaminants on the regulation of the StAR Protein

1670 4-NONYLPHENOL ALTERS STEROID HORMONE METABOLISM IN THE LIVER OF FVB MICE.

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4-Nonylphenol (4-NP) is an endocrine disrupting chemical that enhances proliferation of MCF-7 breast cancer cells due to its affinity for the estrogen receptor. Recent data also indicates that 4-NP binds to the PXR receptor (Masuyama et al., 2000), therefore it has the potential to alter CYP 3A family member expression. These P450s are important in the inactivation and 6β-hydroxylation of testosterone. Because PXR receptor activation appears to alter xenobiotic and steroid metabolism, we hypothesized that 4-NP may alter steroid hydroxylation and possibly steroid conjugation. This may be important in activating 4-NP's own elimination or the elimination of other chemicals; however, it is also possible that 4-NP may alter steroid hormone metabolism in ways that are not beneficial to the organism. Female FVB mice were treated for seven days with 0, 25, 50, and 75mg/kg/day 4-NP in honey, and then euthanized. Livers were excised, microsomes and cytosol prepared and then both estradiol and testosterone glucuronidation, sulfation and hydroxylation was examined. 4-NP significantly increased estradiol sulfotransferase activity 50% at 75mg/kg/day 4-NP and in a dose-dependent fashion, but 4-NP did not increase estradiol or testosterone glucuronyltransferase activity. 4-NP did signficantly inhibit both estradiol and testosterone glucuronyltransferase activity *in vitro*. We are currently investigating 4-NP's actions on estradiol and testosterone hydroxylase activity, and those results will be presented. These alterations in steroid conjugation may effect clearance of steroids from exposed tissues, and thus increase the potential of 4-NP to promote steroidogenic cancers.

1671 STIMULATION OF THE THYROID GLAND WITH THYROTROPIN RELEASING HORMONE IN THE RAT: VALIDATION OF THE MEASUREMENT OF THYROID STIMULATING HORMONE.

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The purpose of this study was to validate the measurement of serum Thyroid Stimulating Hormone (TSH) with a rat specific radioimmunoassay (Amersham, UK) after stimulation with commercial Thyrotropin Releasing Hormone ((TRH UCB® (Protirelin)). T3 (triiodothyronin) and T4 (thyroxine) were measured as well. TRH stimulates the release of TSH by the anterior pituitary which regulates the secretion of the thyroid hormones. Commercial TRH is used for diagnostic purposes in the TRH-test in humans. TRH was given as a single IV injection to 3 groups of five male and female rats each at a dose of 2.5 micrograms/kg body weight. Rats of a fourth, control, group were not injected. Blood was sampled *via* the orbital venous plexus 15 minutes after stimulation in the first group, after 30 minutes in the second and after 60 minutes in the third. Stimulation of each group (and sampling in the controls) was done on three different occasions with 7 days between to check reproducibility. The final blood sampling was done at terminal kill of the animals (pentobarbitone anaesthesia) *via* the carotid artery. Increase in TSH levels reflected the expected biological response after stimulation of the pituitary gland by exogenous TRH. Approximately 15 and 30 minutes after stimulation, an increase in TSH levels occurred in male and female rats. This increase was no longer statistically significant 60 minutes after stimulation in males. In females, TSH levels were comparable or lower than the control group after 60 minutes. Both in male and female rats, stimulation of the pituitary gland with TRH resulted in increased T3 levels at 30 and/or 60 minutes after TRH administration. T4 levels remained unchanged. The stimulation model tested was reproducible in time. Stimulation with commercial TRH can be used to test the integrity of the hypothalamic-pituitary-thyroidal axis in the rat or as an aid in validation of measurement of hormones related to this system.

1672 PERCHLORATE INDUCED THYROID HORMONAL CHANGES IN PREGNANT RATS AND FETUSES.

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The production and storage of ammonium perchlorate (AP) has resulted in the contamination of soil, ground, and drinking water in a number of states. The perchlorate ion interferes with iodide uptake in the thyroid, resulting in reduced thyroidal hormone synthesis. In rodents, this leads to lower triiodothyronine (T_3) and thyroxine (T_4) levels in the blood, and the activation of thyroid stimulating hormone (TSH). A time-course kinetic study with perchlorate was conducted to examine perchlorate kinetics and corresponding hormone changes in pregnant rats. Pregnant rats (n=8) were administered perchlorate at a concentration of either 0 or 0.1 mg/kg *via* tail vein on gestation day 20 (GD20) and sacrificed at 1, 4, 8, 12, 24 and 48h post dosing. Maternal and fetal serum perchlorate concentrations, determined at all time points, reached their maximum at 4 hours after perchlorate exposure. Serum perchlorate concentrations were undetectable at 24 and 48h post dosing in the dams, where as in the fetuses they were undetectable at 8, 12, 24 and 48h post dosing. Serum levels of TSH, T_3 and T_4 were measured in both the dams and fetuses. In the 0.1-mg/kg-dose group serum TSH levels were increased significantly ($p<0.05$) at 4h and 8h post dosing in both the dams and fetuses. Serum thyroid

hormones decreased significantly ($p<0.01$) at 4h post dosing in the 0.1 mg/kg perchlorate dose group in both the dams and fetuses. Maternal and fetal serum TSH levels at 0.1 mg/kg perchlorate dose in the 8h post dosing groups were 5.47 ± 0.34 and 6.06 ± 0.45 ng/mL as compared to maternal and fetal serum TSH levels of 4.60 ± 0.38 and 5.11 ± 0.49 ng/mL in the control groups at GD20. Fetal Serum T_4 levels were significantly decreased at 4h, 8h and 12h post dosing in the 0.1 mg/kg dose group. Assessment of serum perchlorate and thyroid hormone levels serves as the most sensitive end point to understand perchlorate effects during gestation and provide data to develop a pharmacodynamic model for perchlorate in rodents.

1673 EXPRESSION PROFILE IN LIVER, PITUITARY, AND THYROID FROM PHENOBARBITAL TREATED RATS.

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Phenobarbital is a microsomal enzyme inducer that causes thyroid follicular cell hyperplasia and tumors in rats, presumably by increasing the clearance of thyroxine (T4) and triidothryonin (T3) leading to a compensatory increase in serum thyroid-stimulating hormone. Increased clearance of T3 and T4 is due in part to induction of UDP-glucuronosyl transferase (UGT). The induction by phenobarbital in the liver is well documented, however its effects in the thyroid and pituitary have not been reported. Through the use of DNA arrays, the effect of phenobarbital (100 mg/kg/day) on the expression of 8, 740 genes in the liver, thyroid, and pituitary of male rats treated for 14 days was measured. Several xenobiotic metabolizing enzymes, including the CYP 2B and 3A family, as well as UGTs were induced in all three tissues. Specifically, CYP3A1 was induced 3.4, 15, and 14.4-fold, CYP3A3 was induced 2.4, 11.9, and 18.7-fold, CYP2B2 was induced 51.2, 6.7, and 21.4, CYP2B19 was induced 9.8, 27, and 37.3-fold, and UGT2B was induced 4.4, 4.5, and 19.5-fold in rat liver, pituitary, and thyroid, respectively. These findings imply that thyroid hormone metabolism in extrahepatic tissues could contribute to the mechanism of phenobarbital induced tumors in the rat by reducing hormone levels at the site of hormone regulation.

1674 SHORT-TERM EXPOSURE TO AN ENVIRONMENTAL MIXTURE OF PHAHS: DOSE-ADDITIVE EFFECTS ON SERUM THYROXINE.

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Endocrine disruption from environmental contaminants has been linked to a broad spectrum of adverse outcomes. An additional concern about endocrine disrupting xenobiotics is the potential for additive or synergistic effects of mixtures. A short-term dosing model to examine the effects of environmental mixtures on thyroid homeostasis (TH) has been developed. Prototypic chemicals such as dioxins, polychlorinated biphenyls and polybrominated diphenyl ethers have been shown to alter thyroid hormone homeostasis in this model primarily by up regulating hepatic catabolism of thyroid hormones. Current efforts are testing the hypothesis that the effects of mixtures of these chemicals on thyroxine (T4) concentrations can be predicted by dose-additivity theory. The current study used 28 day-old female Long-Evans rats, orally dosed with varying concentrations of 18 different PHAHs (2 dioxins, 4 dibenzofurans, and 12 PCBs, including dioxin-like and non-dioxin-like PCBs) for four consecutive days. Serum samples were collected 24 hours after the last dose. Serum total T4 was measured *via* radioimmunoassay. Extensive (7-9 doses/chemical) dose-response functions were statistically modeled to determine ED50s using the Hill equation. A mixture was custom synthesized with the ratio of chemicals based on environmental concentrations (e.g., relative to TCDD: TCDF-1.5x, PCB126-50x; PCB153-30, 000x). Serial dilutions of this mixture were tested in the 4-day dosing assay. Predicted outcome, based on the assumption of dose-additivity, was tested using statistical dose-response modeling. Preliminary analyses of results suggest that the effects of the mixture on serum total T4 can be predicted by dose-additivity. There was no evidence of synergism or antagonism. Future work will expand the mixture to include chemicals from diverse classes of thyroid disruptors such as TH synthesis inhibitors. *This abstract does not necessarily reflect the policy of the USEPA.*

1675 DDE PARTIALLY RESTORES ANDROGEN-REPRESSED TRANSCRIPTIONAL ACTIVITY OF THE CONSTITUTIVELY ACTIVE RECEPTOR (CAR).

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The constitutively active receptor (CAR) is an orphan nuclear receptor that primarily regulates the transcriptional induction of cytochrome P450 (CYP) 2B but also cross-regulates the CYP 3A gene. Steroid hormones are CAR ligands and affect CAR-mediated regulation of CYP 3A and 2B expression. CYP 3A and 2B are involved in the metabolism of steroid hormones. Xenobiotics have also been demonstrated to activate or repress CAR-mediated transcriptional regulation of enzyme expression. Alterations in CAR-regulated CYP 3A and 2B expression may lead to enhanced steroid catabolism and alterations in endocrine function. The aim of this current study was to test the hypothesis that the antiandrogen 1, 1-dichloro-2, 2-bis(p-chlorophenyl)ethylene (p, p-DDE, or DDE) affects androgen-induced repression of CAR transcriptional activation. Activation of CAR in response to chemical exposure was determined in cultured HepG2 cells that were cotransfected with mouse CAR expression plasmid, a control vector (pRL-SV40), and the luciferase reporter gene construct containing the CAR nuclear receptor binding site NR1. CAR activation was lower in cells treated with testosterone and other androgens than in cells treated with vehicle alone. No significant effect was observed in CAR activation following exposure to DDE alone at concentrations ranging from 0.01 to 50 μM. However, transcriptional activation of CAR was partially restored by DDE in cells treated with androgens. These data indicate that DDE mitigates androgen-induced repression of CAR transcriptional activation and suggest involvement of CAR in the antiandrogenic effects of DDE.

1676 INTERACTION OF TRENBOLONE, A POTENTIAL FEEDLOT EFFLUENT CONTAMINANT, WITH HUMAN AND RAINBOW TROUT ANDROGEN RECEPTORS (AR) *IN VITRO* AND *IN VIVO* ANDROGENIC EFFECTS IN THE RAT.

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Concern has arisen regarding the presence and presistence of trenbolone in the environment. Trenbolone acetate is an anabolic steroid used to promote growth in cattle. It is hydrolyzed to the active compound, 17 beta-trenbolone, which is one of the primary metabolites then excreted by cattle. Both reproductive alterations in fish living in waters receiving feed-lot effluent and *in vitro* androgenic activity in feedlot effluent samples have been reported . In the current study, the potency of 17 beta-trenbolone was examined both *in vitro* and in an *in vivo* screening assay for androgenic activity. In COS cell binding assays using transfection of rainbow trout or human AR, trenbolone competed equally well for [3H]R1881 binding to either AR with relative binding affinities of 4.0 % and 3.4%, respectively, *versus* 100% for R1881. Trenbolone was also tested for its ability to activate androgen dependent transcription at concentrations ranging from 1 pM to 10 mM in MDA-kb2 cells with endogenous human AR and a stably transfected androgen responsive luciferase reporter. Trenbolone was a full AR agonist in these cells producing a Dose-Response curve similar to that of dihydrotestosterone. Futhermore, agonist activity was inhibited by the AR antagonist, hydroxyflutamide. Trenbolone was also a potent AR agonist in the N/C terminal interaction assay performed in HeLa cells, an assay that is highly specific for androgens. When examined *in vivo* in the Hershberger assay, trenbolone displayed selective AR mediated (SARM) activity, affecting some androgen-dependent tissues in rats more than others suggesting that 5 alpha reduction inactivated the parent compound. In summary, trenbolone is a potent SARM. Further studies are needed to determine if trenbolone is present in feed-lot effluent and to characterize the ability of this chemical to alter vertebrate reproduction and development.

1677 POST-TRANSCRIPTIONAL EVENTS ARE INVOLVED IN CYPROHEPTADINE INDUCED INHIBITION OF PREPROINSULIN SYNTHESIS IN RINm5F CELLS.

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Previous studies have demonstrated that the antihistaminic, antiserotonergic drug cyproheptadine (CPH) inhibits insulin synthesis in RINm5F cells without an alteration in preproinsulin mRNA (PPImRNA) levels. This suggests that the compound is acting *via* one or more post-transcriptional mechanisms to inhibit the synthesis of insulin. A critical step in secretory protein synthesis is the translocation of the ribosome-associated nascent chain from the cytosolic compartment, where protein synthesis begins, into the lumen of the endoplasmic reticulum (ER) where translation continues. Using a subcellular fractionation technique followed by real-time RT-PCR, the percentage of PPImRNA in various stages of ribosome association was examined. Control cells showed 62.7 ± 2.0% of PPImRNA in ER-bound form and 31.6 ± 3.4% in translationally-initiated free cytosolic form while a two hour treatment with 10μM CPH altered these distributions to 38.7 ± 3.9% and 52.9 ± 4.9% respectively. CPH also induced an increase in the percentage of translationally-uninitiated free PPImRNA. This CPH induced dislocation of PPImRNA from ER-bound form to free cytosolic forms was found to be concentration dependent, evident within thirty minutes, and reversible upon removal of the compound. Also,

the ability of CPH to induce PPImRNA dislocation was found to be chemical structure specific as the CPH analog 4-diphenylmethylpiperidine, active in inhibition of insulin synthesis, elicited similar PPImRNA dislocation while the CPH analog 2-diphenylmethylpiperidine, inactive in inhibition of insulin synthesis, did not. Overall, these data provide evidence supporting an effect of CPH on PPImRNA translation. CPH may act to inhibit insulin synthesis by altering one or more of the processes involved in the initiation or elongation stages of translation or in the binding of ribosomes to the ER.

1678 AROMATASE INHIBITION BY AZOLE DRUGS: ASSESSING PREGNANCY RISKS.

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Human aromatase (CYP19) converts C_{19} androgens to aromatic C_{18} estrogenic steroids that are important for pregnancy maintenance and also in regulating uterine contractions in late pregancy. Inhibitors of this enzyme are associated with fetal loss in non-human primates. We tested a panel of azole antifungals which may be administered to pregnant women for their potential to inhibit aromatase using the fluorometric substrate dibenzylfluorescein and cDNA-expressed CYP19. Potency varied by several orders of magnitude. For example, metronidazole was consistently inactive as an inhibitor at concentrations up to 100 μM whereas ketaconazole and miconazole returned IC_{50} values of 1.5 μM and 0.04 μM, respectively. Typical plasma levels of some azole drugs (e.g. miconazole) following oral or topical administration are at or above these IC_{50} values. These data suggest that some azole compounds may disrupt estrogen production in pregnancy and may affect pregnancy outcome.

1679 EFFECTS ON AROMATASE ACTIVITY IN MAMMALIAN CELL LINES AFTER EXPOSURE TO VARIOUS PESTICIDES.

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Imidacloprid, carbendazim, epoxiconazol and atrazine are widely used pesticides in Europe and the USA. These compounds contain a triazine, imidazole or triazole ring and previous research suggests that these molecular structures are, at least partly, responsible for interaction with aromatase (CYP19). Interference with the aromatase enzyme can cause an alteration in steroidogenesis, leading to an enhanced or impaired conversion of androgens to estrogens. We used the rat Leydig tumor cell line R2C to assess the effects on aromatase activity after a 24h exposure to these pesticides. Concentrations ranged from 0.3 to 100 mM. Aromatase activity, with a K_M value of 25 nM for androstenedione, was measured using a ^3H_2O-release assay. Cytotoxic effects of the pesticides were assessed by performing an MTT test for cell viability. Exposure of R2C cells to imidacloprid resulted in a 1, 5 fold induction of aromatase activity at concentrations > 30 mM, while no effect on cell viability was observed up to 100 mM. Epoxiconazol and carbendazim showed an inhibitory effect on aromatase activity at concentrations > 3 mM and > 10 mM, respectively. Both pesticides caused a decrease in cell viability at concentrations > 100 mM. Atrazine did not affect aromatase activity nor cell viability in R2C cells, whereas it was shown to cause induction of aromatase activity in the human adrenocortical carcinoma cell line NCI-H295R. These results suggest different mechanisms of action concerning regulation of aromatase activity within the different cell systems. It is concluded that these commonly used pesticides have the potential to interfere with steroidogenesis and could possibly be endocrine disruptors in the *in vivo* situation. Further research on effects of these pesticides and on their mechanism of action concerning regulation of aromatase activity within the NCI-H295R cell line and the rat Leydig carcinoma cell line LC-540 is in progress.

1680 PUBERTAL DEVELOPMENT IN FEMALE WISTAR RATS FOLLOWING EXPOSURE TO PROPAZINE AND ATRAZINE METABOLITES, DIAMINO-S-CHLOROTRIAZINE AND HYDROXYATRAZINE.

S. C. Laws, J. M. Ferrell, T. E. Stoker and R. L. Cooper. *RTD, NHEERL, USEPA, Research Triangle Park, NC.* Sponsor: R. Kavlock.

We have shown that the chorotriazine herbicide, atrazine (ATR), delays the onset of pubertal development in female rats. ATR and its by-products of microbial degradation are present in soil and groundwater. Since current maximum contaminant levels are set only for ATR, it is important to determine whether or not these metabolites can alter pubertal development and possibly pose a cumulative exposure hazard. Here, we have evaluated the effects of two ATR metabolites, diamino-s-chlorotriazine (DACT) and hydroxyatrazine (OH-ATR), and a structurally similar chlorotriazine, propazine (PRO), on female pubertal development. Rats were gavaged from postnatal day (PND) 22 through PND41 with DACT (16.5, 33.7, 67.5, 135 mg/kg), OH-ATR (22.8, 45.7, 91.4, 183 mg/kg), or PRO (26.5, 53, 107, 213 mg/kg). The dose range for each chemical was selected as the molar equivalent of ATR (25, 50, 100, 200 mg/kg). Females were monitored daily for vaginal opening (VO) and killed on PND 41. DACT delayed VO by 3.1, 4.5, and 7.1 days compared to controls (32.7 ± 1.8 days of age) after doses of 33.7, 67.5 and 135 mg/kg, respectively. The NOAEL for DACT (16.5 mg/kg) was identical to the equimolar NOAEL for ATR (25 mg/kg). Body weight (BW) on PND41 was reduced only by the high dose of DACT (7.1% reduction) indicating that growth was not associated with delayed VO. Also, the BW on the day of VO was increased by 33.7, 67.5 and 135 mg/kg DACT. PRO (107 or 213 mg/kg) delayed VO by 4 days, but did not alter BW on PND41. In contrast, the dechlorinated metabolite, OH-ATR, did not significantly delay the age at VO, suggesting that the presence of the chlorine group may be necessary for activity. Together, these data demonstrate that PRO and DACT can delay the onset of puberty in the female rat at doses equimolar to that of ATR, and suggest that environmental exposure to multiple chlorotriazines and metabolites may pose a cumulative hazard. *This abstract does not reflect USEPA policy.*

1681 EFFECT OF AN ACUTE EXPOSURE TO MOLINATE ON OVULATION IN THE RAT.

T. E. Stoker, D. Guidici, S. Jeffay, S. D. Perreault and R. L. Cooper. *RTD, USEPA, Research Triangle Park, NC.* Sponsor: R. Kavlock.

Molinate, a thiocarbamate herbicide used on rice, has been shown to impair fertility in the male rat. In a two-generation study, molinate exposure to female rats resulted in decreased litter size and ovarian abnormalities. However, published data is lacking on the effects of acute exposure to molinate in the female. Based on reported esterase inhibition by molinate and our previous observations with related dithiocarbamates, we hypothesized that a single exposure to molinate during the critical window for the neural trigger of ovulation on proestrus would alter the timing of ovulation. Cycling females gavaged with 0, 25 or 50 mg/kg molinate at 1300h on proestrus were examined for ovulation and oocyte number 24 or 48h later. All controls ovulated at 24h. Molinate at 25 mg/kg delayed ovulation for 24h in 43% of the rats, while 50 mg/kg delayed ovulation in 85%. The number of ova present in the delayed females was unaltered. To examine LH secretion, ovariectomized (OVX) rats were implanted with physiological levels of estradiol (E2) and the induced LH secretion determined by tail bleeds at 0, 2, 4 or 6h after a single oral dose at 1300h. 25 mg/kg molinate caused a significant suppression of the LH in 67% of the rats (4h mean = 0.566 ± 0.1 ng/ml vs 3.560 ± 0.7 ng/ml in controls). 50 mg/kg molinate suppressed the LH surge in 83.3 % of the rats (4h mean = 0.347 ± 0.1 ng/ml). In another study, OVX/E2 rats were killed at 0, 1, 3 and 6h after similar dosing. 50 mg/kg suppressed the LH surge in 75% of the rats, while 25 mg/kg suppressed the LH surge in 63%. These results indicate that molinate is able to delay ovulation by suppressing the LH surge on the day of proestrus and that the brain and/or pituitary are target sites for this pesticide. These effects may explain in part the effects observed in previously published chronic studies with molinate in the female rat. Because delayed ovulation can result in altered pregnancy outcome and reduced litter size, it will be important to determine the precise CNS/pituitary mechanism involved in this effect. This abstract does not reflect USEPA policy.

1682 EFFECTS OF OCTAMETHYLCYCLOTETRASILOXANE (D4) AND PHENOBARBITAL (PB) ON LH SURGE AND OVULATION IN SPRAGUE-DAWLEY (SD) RATS.

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This study was conducted to assess the potential mechanism of previously observed reproductive effects of D4 in SD rats. Cannulated SD rats were exposed to 0, 700, or 900 ppm D4 vapor for 3 days (6 hr/day) from diestrus day-1 through the day of proestrus. A different set of rats received saline or PB (40 mg/kg, i.p.) on the day of proestrus at 1:45 p.m. Blood for the analysis of LH was collected with autologous replacement on the day of proestrus at 2, 4, 6, 8, and 10 p.m. Trunk blood was collected on the day of estrus at 8 a.m. for the evaluation of plasma estradiol (E2) levels in D4- and PB-treated rats. Ova were counted in wet mounts of fresh oviducts. The ovaries were routinely fixed and processed for light microscopy. D4 exposure increased the percentage of rats with attenuated LH surge at 4, 6, and 8 p.m. in 900 ppm group, and at 6 p.m. in 700 ppm group relative to controls. Similarly, the LH surge in PB-treated rats was suppressed at all time points. In D4-treated rats, E2 levels on the day of estrus were higher both in 700 (22.2 ± 4 pg/mL) and 900 ppm (21.1 ± 1.8 pg/mL) groups relative to controls (7.8 ± 1.4 pg/mL, $p < 0.05$). Plasma E2 levels in PB group were also higher (12.1 ± 2.7 pg/mL *vs.* 6.8 ± 2.1 pg/mL in controls), but not statistically significant. D4 and PB both significantly decreased

the percentage of rats that ovulated. Among ovulators, they had little effect on the number of ova produced. Rats that failed to ovulate had numerous mature follicles and lacked basophilic (new) corpora lutea as evidenced by histopathology. Control and treated rats that failed to ovulate had similar hormonal and ovarian morphology profiles; conversely, control and treated rats that ovulated normally were also similar. These results indicate that D4 and PB have similar suppressive effects on LH and ovulation. Supported in part by Silicones Environmental, Health, and Safety Council of North America.

1683 THE EFFECT OF MELATONIN AGAINST RADIATION INDUCED DAMAGE IN ALBINO RATS.

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Melatonin is a hormone produced by the pineal gland and has been shown to have radioprotective properties. The objective of this study was to determine the potential radioprotective effects of melatonin. Adult albino rats were treated with an oral dose of 250 mg/kg melatonin one hour before exposure to whole-body gamma radiation of a single dose 8 Gy (Lethal dose LD50/30). The animals were observed for mortality over a period of 30 days following irradiation. A second group was treated with an oral dose of 250 mg/kg melatonin one hour before exposure to whole-body gamma radiation of single dose 6 Gy (Sublethal dose). Two days post irradiation animals were sacrificed and the following were analyzed: histopathology, lipid peroxidation, bone marrow, blood cytology and biochemical studies. A third group was treated oral doses of 250 mg/kg melatonin one hour before exposure to whole-body gamma radiation of multiple doses of 1 Gy per week for 6 weeks (Fractionated doses). Two days post last irradiation animals were sacrificed and the same analysis performed. The data obtained in this study suggest that melatonin at a dose as high as 250 mg/kg is non-toxic, and that high doses of melatonin are effective in protection of rats from effects of lethal, sublethal and fractionated doses of whole-body irradiation.

1684 PLACENTAL RESPONSES TO BUTADIENE DIEPOXIDE TREATMENT IN PREGNANT RATS.

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Butadiene diepoxide(BDE)is a metabolite of 1, 3-butadiene that is reproductively toxic in rats and mice and is utilized in synthetic rubber production. In rats specifically, since BDE-induced reductions in ovarian weights and follicular growth may result in impaired uterine function *via* steroid regulation, the purpose of the study was to clarify biochemical and physiological responses to post-implantation BDE exposure (gestation days 5-8). Gestation in female Sprague-Dawley rats 220-250g, maintained under 12L:12D and 20+2oC, was initiated by cohabitation between a fertile male and proestrous female rats. Rats (n=6/group) were treated with 5 daily 0900-1000h intraperitoneal doses of BDE 0, 0.25, 0.30, 0.35 or 0.40 mmol/kg in sesame oil. The dosing volume was 2.5ml in sesame oil/kg body weight. Animals were sacrificed on gestation day 9 or 12 0900-1000h, the period of placentation and early fetal development, for retrieval of uterine tissues. Placentae were scraped away from the adjacent uteri. This procedure was followed by bioassay determinations placental PACAP mRNA expression by RT-PCR, placental MMP-9 activity by substrate zymography and serum progesterone levels by RIA; and by developmental evaluations of implantation sites, deaths/resorptions and corpora lutea. The results analyzed by Student's t test, showed that in pregnant BDE-treated rats, there were dose-linked reductions in placental PACAP mRNA $p<0.001$, placental MMP-9 activity ($p<0.001$) and in the number of dead or resorbed fetuses $p<0.001$. These reductions were similar when measured on either gestation day 9 or 12. Declines in implantation sites and corpora luteal levels were more consistent under gestation day 12 than at day 9. However, fetal mortality was drastically increased overall by 100% on gestation day 12 compared to a general increase of 55% on gestation day 9. The data suggest that the pervasive anti-placental biochemical and developmental actions of post-implantation BDE treatment in pregnant rats were apparently more critical as fetal development progressed from gestation day 9 to day 12. Supported by NIEHS/ARCH Research Grant ES 10018-02

1685 INDUCTION OF VITELLOGENIN IN RESPONSE TO GENISTEIN FROM A SOY BASED DIET IN CHANNEL CATFISH.

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Genistein, 4', 5, 7-trihydroxyisoflavone, is a phytoestrogen contained in soy. Soy is a major component of many commercial catfish feeds. Genistein may induce the hepatic production of the egg yolk protein, vitellogenin, normally induced by endogenous estrogen. Channel catfish were fed a specialized diet containing casein as the protein source, and not containing soy, for 18 days to allow dissipation of genistein from the tissues. Channel catfish were injected ip once with .02mg/g 17 β-estradiol, as a positive control, and serum was collected. Vitellogenin was precipitated using N, N-dimethylformamide from serum of catfish treated with .02mg/g 17 β-estradiol. Total protein in the precipitate was determined using the BCA protein assay. Vitellogenin was identified using Western blot and quantified using ELISA with a vitellogenin specific monoclonal antibody. Catfish were gavaged once with 0.2mg/g, 1.0mg/g, or 2.0mg/g body wt. genistein. Serum and liver samples were taken for analysis. A dose-response curve for vitellogenin synthesis was generated from catfish gavaged with genistein. A second group was gavaged with 0.2mg/g or 2.0mg/g genistein to determine the dissipation of genistein in the liver and the effect of genistein on vitellogenin synthesis over time. A third group was placed on a soy-based diet. Levels of genistein in the liver were determined by HPLC. The fish that were gavaged with genistein or placed back on a soy diet showed an increase in genistein in the liver. Soy fed catfish had vitellogenin levels in their serum greater than casein fed catfish. The results suggest that the phytoestrogens present in commercial soy-based catfish food can bind the hepatic estrogen receptor and induce the synthesis of vitellogenin. (Supported by USDA/ARS 58-0790-0-120).

1686 COMPARISON OF DERMAL AND INHALATION EXPOSURES TO JP-8 JET FUEL.

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JP-8 is a kerosene based fuel consisting of a complex mixture of hundreds of components from a number of hydrocarbon classes, including straight chain alkanes, branched chain alkanes, cycloalkanes, diaromatics and napthalenes. Occupational exposure to JP-8 occurs *via* both the inhalation (aerosol and vapor) and dermal routes. It is thus necessary to extend accepted standards (such as the OEL) for permissible levels of JP-8 and other similar materials in ambient air to the dermal exposure route. This can be done by comparing the absorbed doses *via* each exposure route. The dermally absorbed dose can be estimated from the exposed concentration c_{skin}, the exposure time, exposed surface area A and the dermal penetration coefficient K_p. This latter parameter can be estimated from the physico-chemical properties of the chemical (such as its molecular weight and octanol-water partition coefficient). For inhalation exposure, the absorbed dose depends on the concentration c_{air} in the ambient air, the alveolar ventilation rate Q_a, and the fraction f of the material inhaled that is actually absorbed. Assuming f is 100% often leads to a non-conservative and misleading under-assessment of the relative importance of the dermal route. A more realistic estimate of f can be made from the blood-air partition coefficient P_b (which can be measured or estimated from physico-chemical properties), together with its concentration c_b in the alveolar blood. This latter can be estimated, in turn, by means of a PBPK model. However, in most cases, development of such a model is expensive and time-consuming and so is not practical. Nonetheless, useful estimates of c_b can be made under specific limiting conditions of systemic elimination and metabolism. This leads to useful upper and lower limits on the ratio R of skin to lung absorption rates that depend on c_{air} and c_{skin}, as well as K_p, P_b, A, Q_a, and cardiac output Q_c. These limits can be used to assess the relative importance of the dermal and inhalation routes under specific exposure scenarios, as well as to compare dermal exposures with inhalation exposure standards.

1687 FETAL HORMONAL CONDITION IS AFFECTED BY PHYTOESTROGEN TRANSFERRED FROM MOTHER.

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Phytoestrogens are estrogen-like chemicals. Phytoestrogens are suggested to contribute to prevention of chronic diseases. But one concern is that exposure to phytoestrogens may pose a developmental hazard to infants and fetuses. To study whether the exposure to phytoestrogens had any influences on human fetuses, we investigated feto-maternal relationship between the serum concentrations of phytoestrogens and relationship between phytoestrogens and hormonal condition of fetuses. Serum samples were obtained from 20 mothers and umbilical cord of their babies. Informed consent was required from each subject. This study was agreed on by the ethics committee of Chiba University School of Medicine. Serum phytoestrogen concentrations were determined by liquid-chromatography/tandem-mass

spectrometry. Data were expressed as mean (mean-SD to mean+SD) calculated with the log transferred concentrations, then converted to their original scale. In cord serum, mean genistein concentration was 63.1 (30.6-130.0) ng/ml, mean daidzein concentration was 6.8 (2.7-17.5) ng/ml. Equol was detected in only seven samples. We detected that the concentrations of genistein and daidzein were significantly higher in cord serum than in maternal serum. In cord serum, genistein concentration was negatively correlated to SHBG concentration. These data suggested that the phytoestrogen affected the hormonal condition of human fetuses and the response to the phytoestrogen of the fetuses was different from the adults.

1688 THE VALUE OF LACTIC ACIDOSIS IN THE ASSESSMENT OF THE SEVERITY OF ACUTE CYANIDE POISONING.

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Introduction: In pure cyanide poisonings, the correlation between plasma lactate concentrations and the corresponding blood cyanide concentrations has not been addressed. Thus, we tested the hypothesis that the repeated measurement of plasma lactate concentrations could have a confirmatory value in acute pure cyanide poisonings. Materials and Methods: We reviewed the charts of the 11 patients admitted to our toxicological intensive care unit between 1988 and 2000 who had exposures to cyanide. Victims of smoke inhalation were excluded from this study. Ten patients had pre-treatment blood sampling for cyanide and plasma lactate. Nine patients had repeated blood sampling. Treatment included supportive care and hydroxocobalamin + sodium thiosulfate. Blood cyanide and plasma lactate concentrations were measured by a colorimetric assay using microdiffusion and an enzymatic method (Nal: 9 to 18 mg/l), respectively. Results: Before antidotal treatment, the median plasma lactate concentration was 168 mg/dL, the median blood cyanide concentration was 4.2 mg/L. Using Spearman test, there was a significant correlation between plasma lactate and blood cyanide concentrations ($r = 0.74$, $p = 0.017$). Before antidotal treatment, plasma lactate concentration correlated positively with anion gap and inversely with systolic blood pressure, spontaneous respiratory rate, and arterial pH. Blood cyanide concentrations correlated inversely with systolic blood pressure and arterial pH and positively with anion gap and blood glucose concentration. During the course of cyanide poisonings, a plasma lactate concentration > 72 mg/d/L (8 mmol/L) was sensitive (94%) and moderately specific (70%) for a toxic blood cyanide concentration (> 1.0 mg/L). The specificity was substantially improved in patients not receiving catecholamines (85%). Conclusions: The immediate and serial measurement of plasma lactate concentrations is useful in assessing the severity of cyanide poisoning.

1689 CONSEQUENCES OF BRIEF EXPOSURE TO HIGH-CONCENTRATION CARBON MONOXIDE IN AWAKE RATS.

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Exposure to high concentration carbon monoxide (CO) is of concern in military operations. Experimentally, the harmful consequences of a brief exposure to high concentration CO have been poorly described. This study investigated the development of acute CO poisoning in awake male Sprague-Dawley rats (220-380g). Animals were randomly grouped and exposed to either 1000, 3000, 6000, 10000, 12000 or 24000 ppm CO in a continuous air/gas dynamic exposure chamber for five minutes. Respiration was recorded prior to and during exposures. Mixed blood carboxyhemoglobin (COHb) and pH were measured before and after exposure. Compared to the pre-exposure control, at the end of exposure in the 10000, 12000, and 24000 ppm groups, breath rate was significantly decreased by 64%, 47%, and 18%, respectively. Tidal volume was significantly increased by 121, 137, and 142%, and minute ventilation volume was significantly decreased by 77, 64, and 26%, respectively. Blood COHb was elevated to 60.1, 63.4, and 69.3%, and blood pH was reduced to 7.43± 0.09, 7.25± 0.05, and 7.13± 0.04, respectively. The mortality was 1/12 in the 12000 ppm group and 4/12 in the 24000 ppm group. Deaths occurred in the fifth minute of exposure. Breath rate was < 27% of the control, pH < 6.87, and COHb > 80% in animals that died during exposure. There were no significant changes in respiratory parameters and pH, and no deaths, in rats exposed to CO at concentrations under 6000 ppm, although COHb was elevated to 14.5, 29.7, and 54.3% in the 1000, 3000, 6000 ppm groups, respectively. These results suggested that brief exposure to CO at concentrations under 10000 ppm may cause minor to moderate health risk. However, exposure to CO at concentrations greater than 10000 ppm for a period of time as short as five minutes will change breathing patterns which may result in acute respiratory failure, acidemia, and even death.

1690 AN OUTBREAK OF HEPATITIS AS A RESULT OF OCCUPATIONAL EXPOSURE TO SOLVENTS IN A HAZARDOUS WASTE FUEL BLENDING FACILITY.

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This is a multi-disciplinary investigation of an outbreak of hepatitis. The methods include environmental and occupational health investigations, epidemiological survey, clinical examination, liver function tests, liver biopsy, serological tests for hepatitis A, B, and C, and serum infrared and/or mass spectrometry. Of the 91 workers tested, 44% exhibited symptoms of hepatitis consistent with exposure to chlorinated solvents. These symptoms include nausea, vomiting, abdominal pain, diarrhea, headache, dizziness, fever, and weight loss. Alanine aminotransferase (ALT) levels higher than 1.5 times normal were regarded as case. Among the cases 15% tested positive for hepatitis B or C. A total of 92% of the cases worked in the tower where the fire and clean up occurred. All maintenance workers participating in repair and clean-up had elevated ALT. The liver biopsy of the worker with the highest ALT (8, 000 IU) indicated diffuse hepatitis with acidophilic bodies and microvesicular fat on oil red O stain. Among the workers not normally working in the tower, only 10% were cases. After comparing the medical history, test results, primary work locations, job descriptions, onset of symptoms, usage of PPE among the workers, and other health and safety measures, it is concluded that the outbreak of hepatitis probably resulted from exposure to solvents during the post-fire cleanup. The identification of chloro/fluorocarbons by serum infrared spectroscopy in one case, and the presence of fluorinated compounds in the fire retardant surfactant suggests that chloro/fluorocarbons may have been liberated leading to inhalation exposure as a result of heat from the fire.

1691 COMPARATIVE OCCUPATIONAL EXPOSURES TO FORMALDEHYDE RELEASED FROM INHALED WOOD DUSTS *VERSUS* THAT IN GASEOUS FORM.

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Particle boards and other wood boards are usually made with formaldehyde-based resins. Wood workers are thus exposed to formaldehyde in gaseous form as well as from airborne dust once it enters their respiratory tract. These workers remain exposed to formaldehyde released from the dust still present in their upper respiratory tract, even after their work shift. In assessing the risk associated with formaldehyde exposure, one needs to consider the relative importance of these two sources of exposure. This study proposes a kinetic model to estimate the exposure to formaldehyde released from dust and compares it to exposure from the gaseous form. Model parameters are determined using data from published studies which estimate amounts of dust deposited in different regions of the respiratory tract, their residence time according to particle sizes and the rate of formaldehyde released from wood product dust once in the tract. The model predicts the amount of formaldehyde absorbed by the respiratory tract upon its release from wood product dust for various exposure scenarios. Based on a daily work shift of 8 hr, it is found that, for a dust concentration in air of 5 mg/m^3 and a formaldehyde concentration of 9 µg/mg of dust, the amount of absorbed formaldehyde released from wood dust is approximately 1/100 of the amount absorbed from the gaseous form at a concentration level of 0.38 mg/m^3 (0.3 ppm). Considering that the formaldehyde concentration in wood dust used above is much higher than usually observed and that dust and gaseous form formaldehyde concentrations used are of the order of the recommended threshold limit values, the simulations show that the formaldehyde exposure from wood dust is comparatively negligible.

1692 ASSESSMENT OF TOLUENE AND ETHYL ACETATE EXPOSURE FROM WEARING A COSTUME MASK BONDED WITH CONTACT CEMENT.

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A series of experiments was performed to assess the solvent evaporation rates and relevant exposure concentrations for primary solvents (toluene and ethyl acetate) emitted from a contact cement used to bond foam surfaces within a costume mask (a furry bear head)that is worn by theatrical dancers. Four coats of the contact cement were applied to each of four 3 by 3 inch areas on both foam components of

the mask (the fitted helmet and the mask structural foam); each coat was allowed to dry for 10 minutes before bonding (joining the cement-coated foam surfaces). Solvent concentrations were measured within the mask under undisturbed (static) conditions at 2, 4, 8, 16, and 20 hours after bonding. Each sample was collected as a one-minute grab sample using 1 liter Summa canisters, and analyzed by EPA Method TO-14. An exponential loss pattern was observed for both toluene and ethyl acetate concentrations inside the mask. Ethyl acetate levels diminished quickly and were nondetectable (< 1 ppm) at 8 hours, while toluene levels diminished quickly but were stable at 11 to 14 ppm when measured at 8, 16 and 20 hours after bonding. Personal toluene exposure was simulated at 21 hours after bonding by fitting the mask on an adult-size mannequin equipped with a breathing zone sampling port in a convection chamber at 37 C. Toluene exposure concentrations in the breathing zone ranged from 1.7 to 2.9 ppm when measured at 0, 5, 10, 15 and 20 minutes after donning the mask, and a level of 7 ppm was observed at 30 minutes. These studies illustrate that the majority of solvents in contact cement were evaporated from the costume mask under static conditions within 4 to 8 hours after bonding, that residual toluene levels after that time are relatively low, and that donning the mask displaces most of the residual toluene. Toluene exposures at 1 to 7 ppm are well below occupational exposure limits and adverse effect levels in humans.

1693 AMMONIA EXPOSURE AND DOSE-RESPONSE ASSESSMENT FOR HOUSEHOLD CLEANING USES.

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There is scant information pertaining to airborne ammonia exposures from common household use of ammonia-containing floor/tile cleaners and spray-on glass cleaners. We assessed instantaneous and event-specific time-weighted average (TWA) exposures to airborne ammonia during mixing and use (cleaning with a soaked sponge) of a typical household floor/tile cleaner containing 5% ammonium hydroxide. One cup of the ammonia cleaner was mixed with two gallons of hot water to make the washing solution, as per the label directions. Airborne ammonia sampling was conducted while the tile walls and floors within three different size bathrooms in a residence were washed. We also assessed exposures during use of a spray-on glass cleaner containing 1 to 3% ammonium hydroxide, while washing several large windows in a small office setting. In each case, TWA ammonia was assessed using a breathing zone personal sampling pump equipped with a silica gel tube and analyzed by NIOSH Method 6015. Simultaneous measurements were recorded each minute with a breathing zone probe from a Draeger PAC III monitor with electrochemical cell detector. Peak ammonia levels ranged from 16 to 28 ppm and short-term TWA concentrations ranged from 9.4 to 13 ppm during mixing and cleaning in each the three bathrooms. Ammonia exposures while using spray-on window cleaners were below reliable detection limits (monitor < 3 ppm; TWA < 1.3 ppm). Brief, moderate exposure levels for airborne ammonia can occur during standard bathroom cleaning uses, i.e., averaging 11 ppm. Peak ammonia concentrations were above the 8-hour TWA occupational exposure limit (OEL) of 25 ppm. Use of the concentrate or less dilute cleaning solutions could result in ammonia exposures exceeding the OEL ceiling limit of 50 ppm, and could cause moderate eye, nose and throat irritation and respiratory symptoms in sensitive individuals. Thus, ammonia-containing cleaner products should contain clear warnings to avoid use of undiluted concentrates.

1694 EXPOSURE OF CHILDREN AND ADULTS TO TRANSFERABLE RESIDUES OF CHLORPYRIFOS FROM DOGS TREATED WITH FLEA CONTROL COLLARS.

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Twenty-four households were chosen that included a pet dog and a child between the ages of 3 and 12 years. These family units were used to determine the levels of insecticide that could be transferred by a flea control collar from a pet dog and any increased amounts of chlorpyrifos metabolites in the urine of one child and one adult in the household. Commercially available flea control collars containing the organophosphate insecticide chlorpyrifos (8%, 2.54g) were applied to dogs as per package directions. Transferable residues were quantified by petting the dog in 3 areas (back, neck, neck with collar) with cotton gloves for 5 min and analyzed by GC/ECD. The averaged transferable residues of the insecticide chlorpyrifos were highest from rubbing directly over the collar (168-454 µg), less from the fur of the neck under the collar (49-252 µg), and lowest from the fur of the back distant from the collar (3-14 µg). The urinary metabolite 3, 5, 6-trichloro-2-pyridinol (TCP) was quantified in first morning void samples using a magnetic particle/antibody assay. The urine of both adults and children obtained before the collar was placed on the dog showed measurable levels of TCP, presumably because of the numerous approved agricultural and residential uses of chlorpyrifos. Ranges of urinary TCP levels in the adults and children prior to placement of the flea collar on the pet dog were 1.1-35.0 and 1.9-22.4 ng/ml, respectively. There were no statistically significant increases in urinary TCP in either the adults or the children of the study at any time point up to 4 months after the application of the collar. The range of levels in the adults and children after application of the flea collar to the dogs were 1.4-52.0 ng/ml and 1.2-52.4 ng/ml, respectively. These results suggest that the insecticide residues obtained from the routine use of a flea collar did not contribute significantly higher levels of exposure to the people in contact with pet dogs. (Supported by EPA R 825170).

1695 MEASURING AND ASSESSING THE RISK OF CHEMICAL EMISSIONS FROM CONSUMER PRODUCTS; SUMMARY STUDY OF OFFICE PRINTERS.

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Good product stewardship as well as regulatory and non-regulatory programs necessitates knowledge of high and low-level chemical emissions from consumer products, and understanding their risks to users. This requires a complete IAQ exposure effects assessment that includes multiple components. This study summarizes the results of the assessment of over 100 office printers. Testing was conducted under environmentally controlled chamber conditions with parameters appropriate for the intended use of the product. A sensitive sampling and analysis method with a specialized chromatographic and mass spectral indoor air library was used to properly identify all emissions. Because accurate emission rates are needed to predict consumer exposures for comparison to specifications set by government and private organizations, product-specific emission profiles were fit to appropriate mathematical models. The predicted user exposure concentrations were then compared to multiple databases containing information on inhalation health risks and irritation/odor potentials of all of the chemicals identified in the product emissions. The most common emissions from laser printers were styrene, xylene, and ethylbenzene, while the most common emissions from plotters were acetic acid, benzyl alcohol, and nonanal. These types of compounds are fairly typical in the indoor environment, and the exposure levels contributed by these emissions are low relative to normal indoor levels. However, the compound list is different when examined in terms of highest average emissions. For laser printers they are butyldigol (emission rate = 11, 305 µg/hr), cyclohexane (6, 055 µg/hr), and 2, 2, 4, 4-tetramethylpentane (3, 500 µg/hr), while for plotters they are 2-propanol (19, 500 µg/hr), ethanol (8, 000 µg/hr), and 1, 3-dichloro-2-propanol (3, 800 µg/hr). Exposure concentrations of these and hundreds other chemical emissions were calculated based on a typical use scenario, and then compared to multiple databases to determine health risk, discomfort levels, sensory irritation, and odor perception.

1696 ESTIMATION OF DIETARY INTAKE OF INORGANIC ARSENIC IN CHILDREN.

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Arsenic is a natural component of our environment, and is ubiquitous in soils and in the diet. Accurate dietary intake estimates for inorganic arsenic are needed to establish background levels of exposure for children. Previous investigations have estimated dietary intake in adults in the USA ranging from 1 to 20 µg/day with an average of 3.2 µg/day, based on a comprehensive market basket survey in which 40 commodities anticipated to provide at least 90 percent of dietary inorganic arsenic intake were analyzed for inorganic arsenic content (Schoof et al. 1999a, b). Four samples of each commodity were collected. Total arsenic was analyzed using an NaOH digestion and inductively coupled plasma-mass spectrometry. Separate aliquots were analyzed for arsenic species using an HCl digestion and hydride atomic absorption spectroscopy (Schoof et al. 1999a). In addition, intake in the USA was previously estimated using a more limited number of foods analyzed for inorganic arsenic (Yost et al. 1998), to derive average estimates of 9.4 µg/day in children and 12.7 µg/day for adults. This paper estimates average dietary intake in children using the more robust dataset for inorganic arsenic and the total arsenic dietary intake estimates compiled by FDA. Dietary intake in young children is highly influenced by intake from dairy products, due to the fraction of the diet comprised by dairy products, which contain a relatively high proportion of inorganic arsenic (49%) (Yost et al. 1999, Schoof et al. 1999a). Application of total arsenic intake data from FDA for the years 1982 to 1991, with recent inorganic arsenic percentages resulted in an estimate of average dietary inorganic arsenic intake for children (ages six months to two years) of 6.7 µg/day. Because inorganic arsenic in the diet is naturally occurring and exposure unavoidable, this intake estimate provides a useful context in risk management of arsenic exposure.

1697 RISK CHARACTERIZATION USING DIFFERENT ASSUMPTIONS REGARDING SPECIATED ARSENIC IN FISH AND SHELLFISH.

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When only total arsenic tissue concentrations are available, assumptions are required regarding concentrations of speciated arsenic in fish and shellfish. Using consumption data from the Pacific Northwest, this work demonstrates the effect of different assumptions using tissue data for total arsenic in fish and shellfish recently collected for freshwater and saltwater species in the same area. The results of a worldwide literature search provide the basis for initial assumptions. Additionally, assumptions based on a small amount of speciated arsenic data from the Pacific Northwest are included. Total arsenic concentrations are higher in saltwater fish then in freshwater fish. However, the percentage of inorganic arsenic (As_{inorg}) is higher in freshwater fish than in saltwater fish. Based on sparse information, it appears that lower trophic-level fish tend to have higher percentage As_{inorg}. Total arsenic concentrations tend to be higher in shellfish than in finfish, with a great deal of species variability. The percentage of As_{inorg} in shellfish is higher than in finfish, as is the percentage of another toxic arsenical species, dimethylarsinic acid. Uptake of arsenic in an arsenic-contaminated system appears to be a complex process, and no generalizable trends were observed in the data. In an evaluation that assesses risks from multiple contaminants in the same animal species, the impact of the percent As_{inorg} depends on the overall contaminant concentration present in the species. The importance of the amount of As_{inorg} in animal tissues increases as overall contaminant concentrations decrease. While having speciated arsenical data is preferable in all situations, it is most critical when total contaminant concentrations are suspected to be low. Use of speciated arsenic data facilitates risk management decisions by avoiding risk characterizations in which As_{inorg}, based on assumptions rather than measurements, predominates the risk.

1698 ESTIMATION OF INORGANIC ARSENIC INTAKE FROM FISH: MARKET BASKET *VS.* RECREATIONAL CATCHES.

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Seafood contributes the greatest fraction of total As to the diet; however, the majority of the As in fish is composed of complex organic arsenicals. The contribution of fish to dietary intake of the more toxic inorganic As (As_i) is pertinent both for assessing background As exposures and for assessing the contribution of As in recreationally caught fish to As exposures from surface water. Due to the expense and difficulty of analyzing As_i concentrations in fish, most available fish data are for total As (As_{tot}) only. Dietary intake estimates for Asin fish are frequently made by assuming that a certain fraction of the As_{tot} reported in the fish is As_i. For example, NRC (1999) assumed that 10 % of seafood As is inorganic. Limited data has been available to support such assumptions, but several recent studies suggest that the NRC (1999) assumption may be too high. Schoof, *et al.* (1999) report data for saltwater finfish, canned tuna, shrimp and freshwater fish purchased at markets. From these data the fraction of As_i *vs.* As_{tot} may be estimated. Whereas 2-8 % of freshwater fish As is inorganic, <0.1 to 0.6 % of the marine food As is inorganic. The As_i in all of these samples was very low, so the variation in fractional inorganic content is almost solely due to variations in the amount of As_{tot} reported. Buchet. et al. (1994) reported somewhat higher As_i content in marine fish, ranging from 0.4 to 3 %. Since most seafood intake in typical diets is from marine sources, these and other recent studies suggest that the NRC assumption is an overestimate, and that As_i is closer to 1 % of As_{tot} in seafood. New data also allow estimates of fractional As_i in wild freshwater fish. EVS (2000) report As_{tot} and As_i for fish caught in the Willamette River in Oregon. No As was detected in pike minnow, while the fraction of As_{tot} that was As_i averaged 5 %, 4% and 13 % in bass, carp and sucker, respectively. The implications of these findings for risk assessment of As in fish in surface water and the derivation of ambient water quality criteria for As will be explored.

1699 DOSE RECONSTRUCTION OF BENZENE EXPOSURE FOR PLIOFILM COHORT (1936-1976) USING MONTE CARLO TECHNIQUES.

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Regulatory standards or guidelines for occupational exposure to benzene are based predominantly on studies of workers. The cohort of rubber hydrochloride (Pliofilm) workers, in particular, serves as the primary basis for the OSHA Permissible Exposure Limit (PEL) and ACGIH Threshold Limit Value (TLV). Previous assessments of this cohort by Rinsky (1981), Crump and Allen (1984), and Paustenbach et al. (1992) have relied on varying assumptions about workplace practices and processes over time, as well as different exposure data and assumptions, thereby yielding significantly different estimates of benzene dose for certain job categories. Given the inherent limitations and uncertainties involved in estimating historic exposures for this cohort, a "distributional" rather than a "point estimate" approach should be more informative for this cohort. In this paper, we re-evaluate the Pliofilm data using Monte Carlo techniques and discuss the impacts of various exposure parameters on estimated doses. A key objective of this work is to address criticisms that previous exposure parameter values were either too high or too low. Estimated benzene doses are reported as equivalent 8-hour time-weighted averages for more than ten job categories from 1936 to 1965 (Akron I and II facilities) and 1940 to 1976 (St. Mary's facility). The key sources of uncertainty addressed in the analysis include characterization of benzene air concentrations (background and peak), uptake of benzene from dermal exposures, engineering controls over time, extended work hours and plant shut down in early years, accuracy of monitoring devices, and effectiveness of personal protective equipment. Sensitivity analyses are used to illustrate areas of uncertainty that have the greatest impact on estimated benzene dose. The results of this analysis provide a more thorough and defensible characterization of benzene exposures among the Pliofilm cohort than those previously reported. These results may also be used, in conjunction with the available mortality data, to provide a more refined assessment of benzene's chronic toxicity in humans.

1700 RATE OF HEXAVALENT CHROMIUM REDUCTION BY HUMAN GASTRIC FLUID.

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Hexavalent chromium [Cr(VI)] is reduced to the essentially non-toxic trivalent state [Cr(III)] in the human stomach. This study investigates the rate of Cr(VI) reduction under a variety of simulated gastric conditions, and in real human gastric juice collected from fasting volunteers. The rate of Cr(VI) reduction in simulated gastric fluid was measured at pH values from 1.5 to 4.5, at starting Cr(VI) concentrations ranging from 100 to 400 ppb, in diluted stomach fluid (10-fold), following ingestion of Rolaids®, and in the presence of food. Stomach conditions were simulated by preparing an aqueous mixture of the primary components of gastric fluid (pepsin, mucine, gastric lipase, gastric amylase, etc.), acidifying with HCl, and providing continuous stirring and heating to approximately 37°C. All experiments were conducted for 60 minutes, with Cr(VI) measured using a Hach AccuVac ChromaVer3 instant test kit. The findings of these studies are: 1) real human stomach fluid has a 10-fold greater capacity to reduce Cr(VI) than simulated stomach fluid; 2) within the first 2 to 5 minutes, 0.3 to 1 mg Cr(VI)/L is reduced in real stomach fluid collected under fasting conditions; 3) increasing gastric pH from 1.5 to 4.5 reduces the rate and extent of Cr(VI) reduction by at least one-third; 4) the amount and rate of Cr(VI) reduced is constant regardless of the starting Cr(VI) concentration, which suggests that these results may be extrapolated to lower concentrations of Cr(VI) in drinking water; 5) antacids dramatically increase the pH of the stomach (from pH 1.5 to 8.2) but appear to have little effect on the Cr(VI) reduction rate or capacity as compared to reduction at pH 4.5; and 6) the presence of food substantially increases Cr(VI) reduction in simulated stomach fluid, with 10 mg/L reduced within the first 4 minutes. These results suggest that ingested Cr(VI), at the federal MCL of 100 ppb, would exist in the stomach, even under fasting conditions, for less than 1 minute before being reduced entirely to Cr(III).

1701 EXACERBATION OF UROPORPHYRIA DEVELOPMENT IN MICE HOMOZYGOUS FOR GENETIC DISRUPTION AT THE HEMOCHROMATOSIS (HFE) GENE.

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Two recently developed strains of genetically modified mice have made it possible to study the contribution of mutations at the hemochromatosis (Hfe) and uroporphyrinogen decarboxylase (URO-D) genes to porphyric susceptibility. Homozygosity for the null mutation of the URO-D gene (URO-D-/-) was lethal. Homozygosity for the null mutation of the Hfe gene caused high levels of iron to accumulate in the liver; heterozygosity was without effect. Neither URO-D nor Hfe gene disruption alone was sufficient to cause the development of uroporphyria. We have investigated polyhalogenated aromatic hydrocarbon-induced uroporphyria in male and female mice bearing either or both these genetic variables. In mice heterozygous for a null mutation at the URO-D gene, mild uroporphyria develops 21 days after a single dose of Aroclor 1254, a polychlorinated biphenyl mixture. The presence of high hepatic iron-load from a disruption of the hemochromatosis gene in addition exacerbates the porphyric response. The hepatic porphyrin

levels were higher in female mice. Mice genetically normal at the uroporphyrinogen decarboxylase gene locus do not develop uroporphyria with Aroclor 1254 administration and high iron concentrations in the liver due to disruption of the hemochromatosis gene were without effect. Elevated hepatic porphyrin concentrations, were always accompanied by depressed uroporphyrinogen decarboxylase activity determined in subsequently isolated cytosol, and upon heat denaturation of that cytosol, the presence of a factor that inhibits recombinant human uroporphyrinogen decarboxylase enzyme. Overall, hepatic siderosis from Hfe gene disruption in mouse models of porphyria cutanea tarda was insufficient to cause uroporphyria, but exacerbated its development in mice made susceptible through genetic depression of constitutive URO-D activity.

1702 DISRUPTION BY ORTHO-SUBSTITUTED POLYCHLORINATED BIPHENYLS OF SPONTANEOUS CATECHOLAMINE RELEASE FROM BOVINE CHROMAFFIN CELLS.

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Ortho-substituted non-coplanar polychlorinated biphenyl congeners have been shown to perturbate neurohormonal release mechanisms in a variety of different cells. In dissociated cultures of chromaffin cells from the bovine adrenal medulla 2, 2', 4, 4'-tetrachlorobiphenyl (PCB47) has been shown to enhance the amount of catecholamines spontaneously released from chromaffin cell cultures as measured by high precision liquid chromatography (HPLC) of the culture supernatants. To further elucidate the mechanisms by which ortho-substituted polychlorinated biphenyls could have increased spontaneous release of catecholamines from chromaffin cells single cell carbon fibre microelectrode amperometry was performed in chromaffin cell cultures. These were exposed to PCB47 solubilized in cell culture medium using 0.1% DMSO for 5 h whereas culture medium with 0.1% DMSO added served as control. Carbon fibre microelectrodes amperometry was used to measure the currents due to oxidation of spontaneously released catecholamine quanta. 5 h exposure of chromaffin cells to PCB47 increased the half width, decay time, and frequency of catecholamine current spikes released spontaneously. Latrunculin B, a marine sponge toxin known to depolarize actin filaments and applied in a concentration of 5 µm had a similar action on catecholamine release. However after pretreatment of chromaffin cells using PCB47 Latrunculin B was uneffective to increase the amount of catecholamine release from chromaffin cells as measured by HPLC of the culture medium. The same was true if Cytochalasin D, another well known depolymerizer of actin filaments, was used. From these experiments it is suggested that PCB47 as well as Latrunculin B and Cytochalasin D may interact at a common target side to disrupt the spontaneous release of catecholamines from chromaffin cells. This target side seems to be the actin filament network which exists in the submembraneous area of chromaffin cells regulating the access of chromaffin granula to their membraneous release sites. (Supported by DFG, Germany)

1703 GLUCURONIDATION OF HYDROXYLATED POLYCHLORINATED BIPHENYLS.

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Polychlorinated biphenyls (PCBs) are metabolized to hydroxylated compounds. While many of these metabolites are further converted to either the glucuronic acid or sulfate conjugates by phase II enzymes, which facilitates their excretion, some hydroxylated PCBs persist in the body, reflecting their inability to be conjugated. A possible role of uridine diphosphate glucuronosyl transferase (UDPGT) in the elimination of hydroxylated metabolites of PCBs was investigated. Glucuronidation studies of some PCB metabolites, including ones which are eliminated with relative ease and also ones which are reported to be retained in blood, were carried out. Liver microsomes, prepared from male Wistar rats treated by intraperitoneal injections of phenobarbital for 3 days (400 µmol/kg/day), were used as the source of UDPGT. Enzyme kinetics (V_{max} and K_m) were determined for each of the metabolites. The enzyme efficiency towards glucuronidation (K_{cat}/K_m)was found to be dependent on the structure of the metabolites. Substitution of chlorine atoms on the non-hydroxylated ring greatly lowered the V_{max} of the enzyme, with substitution in the meta- and para- positions being least favorable for enzyme activity. Steric hindrance around the hydroxyl group by chlorines on adjacent carbon atoms did not play a major role. Also, no apparent correlation between the calculated dihedral angle (planarity) and enzyme activity was determined ($r^2 < 0.5$) however, a weak correlation for the surface area of the molecule and partition coefficient was observed ($r^2 \geq 0.5$). (Supported by P42 ES 07380).

1704 ENZYMATIC OXIDATION OF PCB METABOLITES INCREASES INTRACELLULAR OXIDATIVE STRESS.

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Polychlorinated biphenyls (PCBs) are highly persistent environmental contaminants. In rodents PCBs are complete carcinogens. Questions remain concerning the mechanism(s) of PCB carcinogenesis. PCBs are metabolized to mono- and dihydroxylated compounds. *in vitro* the dihydroxylated compounds are further metabolized either slowly by autooxidation or rapidly in the presence of enzymes like peroxidases or prostaglandin synthase to quinones, accompanied by the generation of reactive oxygen species (ROS). In this study, we wanted to evaluate the effect of enzymatic oxidation of PCB metabolites in cells in culture in terms of ROS production. Three human cell lines with different metabolic activities were chosen for this analysis: the leukemia cell line HL-60 (high in myeloperoxidase), the breast cancer cell line MCF-7 (high in prostaglandin synthases) and the lymphoma cell line Jurkat (low levels of either enzyme). We used the hydroquinone and para-quinone of 4-chlorobiphenyl (4ClPh-HQ and 4ClPh-pQ, respectively) as test compounds. Intracellular ROS production was determined using dichlorofluorescein-diacetate (DCFH-DA) that is converted by cellular esterases to DCFH and further in the presence of ROS to the fluorescent DCF, which can be detected by fluorometry, flow cytometry or confocal microscopy. Propidium iodide was used to measure viability. 4ClPh-pQ caused a dose-dependent increase in fluorescence in all three cell lines at sub-lethal concentrations. With 4ClPh-HQ, however, treatment of MCF-7 and HL-60 cells produced an increase in intracellular fluorescence while it had no effect on Jurkat cells. Cells were mostly viable in all treatments with the exception of HL-60 cells in the presence of ≧10 mM 4ClPh-pQ. These results show that PCB quinones can produce intracellular oxidative stress in any cell type. On the other hand, the conversion of PCB-hydroquinone to quinone and hence ROS production occurs at a higher rate only in cell types with high amounts of peroxidases or prostaglandin synthase (Supported by ES 07380).

1705 EFFECT OF PCB 77 ON TOTAL SELENIUM AND SELENIUM-DEPENDENT GLUTATHIONE PEROXIDASE IN RAT LIVER TISSUES.

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Selenium (Se) provides defense against oxidative damage and is a critical component of several proteins including glutathione peroxidase (SeGPx), a cellular antioxidant enzyme. Persistent inducers of cytochrome P450, such as the polychlorinated biphenyls (PCBs), may generate reactive oxygen species and are associated with oxidative stress. Among the PCB congeners, PCB 77 (3, 3', 4, 4'- tetrachlorobiphenyl) is an aryl hydrocarbon receptor (AhR) agonist, and an efficacious inducer of cytochome P450. The effect of PCB 77 on hepatic total Se and SeGPx activity in male and female rats was investigated. We administered PCB 77 dissolved in corn oil (300µg/kg i.p. bolus dose) to male and female Sprague-Dawley rats fed with unrefined rat chow. The animals were sacrificed at different time points after dosing: 6 hours, 12 hours and 1, 4, 7, 14 and 21 days. Cytochrome P450 1A1 was induced in all treated animals. Hepatic total Se levels was decreased in the 4 to 21 days treatment groups of female rats. In contrast, there is no marked reduction in the treated male rats. Hepatic, cytosolic Se-GPx activity was also diminished in the 4 to 21 days time points for both male and female with the 3 week time point reduced by 6 times. However, the female SeGPx activity was markedly higher than the male in all time points except for the 12 hour time point. A study of GPx activity in vehicle (corn oil-treated) rats found that GPx activity in the liver were higher in female than male rats. This indicates that female rats may be more sensitive to PCB 77 effects on Se. Gender also appears to influence PCB 77's associated effects on the SeGPx enzymatic activity. (Supported by ES 07380)

1706 DNA ADDUCT FORMATION BY POLYCHLORINATED BIPHENYLS: CROSS-SPECIES COMPARISON OF MICROSOMAL BIOACTIVATION SYSTEMS AND ADDUCTION BY SYNTHETIC QUINOID METABOLITES.

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PCBs are ubiquitous environmental contaminants. They are complete carcinogens in rodents and DNA adduction by PCBs was reported. The current study was designed to compare DNA adducts formed after bioactivation of PCBs with rat, mouse and human hepatic microsomes, and to investigate the role of quinoid PCB metabolites in DNA adduction. Eight congeners ranging from mono- to hexachlo-

rinated biphenyls were tested. Metabolites obtained through microsomal bioactivation as well as synthetic quinoid metabolites of 4-chlorobiphenyl were incubated with calf-thymus DNA. The resulting adducts were analyzed by ^{32}P-postlabelling. Adducts were formed only with mono- di- and trichlorinated congeners. Similar adduct patterns were observed with 2-chlorobiphenyl activated with microsomes from all species, while 4-chlorobiphenyl, 3, 4-dichlorobiphenyl and 3, 4, 5-trichlorobiphenyl showed similar adduct patterns in two out of the three systems. 4, 4'-dichlorobiphenyl showed different adduct patterns in all systems. High levels of DNA adduct were formed by the most oxidized quinoid metabolite [2-(4'-chlorophenyl)-1, 4-benzoquinone] and lower levels by the reduced form [2-(4'-chlorophenyl)-1, 4-hydroquinone]. When adducts formed by synthetic quinoid metabolites were compared with those derived from microsomal activation of 4-chlorobiphenyl by co-chromatography, one adduct co-migrated in three different chromatography systems. This study demonstrates that rodents and human hepatic enzymes metabolize lower chlorinated biphenyl congeners to reactive intermediates that form DNA adducts *in vitro* and shows that the *para*-quinone metabolites of PCBs are involved in direct DNA adduction in microsomal systems. (Supported by P42 ES 07380)

1707 THE EFFECTS OF PURIFIED *VS.* UNREFINED DIETS ON LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES IN TESTES OF PCB-153-TREATED RATS.

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The testes are known to be highly sensitive to a number of physical stresses. Among others, reactive oxygen species (ROS) have been shown to impair testicular function. Previous observations led us to postulate that different diets may influence oxidative stress levels in the testes. In this study we compared the effects of purified *vs.* unrefined diets on 1) the production of thiobarbituric acid-reactive substances (TBARS) in the testes as an indicator of oxidative stress, and 2) the activities of antioxidant enzymes in the testes. Thirty-six male Spraque-Dawley rats were fed a purified diet (similar to the AIN-93M diet) or an unrefined diet (Teklad Global 18% Protein Rodent Diet) for 3 weeks. Six animals per diet then received a single IP injection of 2, 2', 4, 4', 5, 5'-hexachlorobiphenyl (PCB-153, 300 µmol/kg), while six other animals per diet received no injection (control) or the vehicle only (corn oil). All animals were euthanized 2 days after treatment, the testes were excised, and subcellular fractions were prepared by differential centrifugation. The results indicate that feeding a purified diet to rats produced a significant decline in TBARS in testes cytosols in control and vehicle groups, and in animals treated with PCB-153, compared to animals on unrefined diets. No reduction in TBARS was seen in testes homogenates. Catalase activity in testes was increased in control groups receiving purified diet compared to unrefined diet, but not in PCB-treated groups. Glutathione transferase activity gave mixed results and total glutathione peroxidase activity was unchanged. This study shows that purified diets may lower oxidative stress in rat testes and that catalase activity may be partially responsible for this decrease. Further studies are needed to identify those dietary components responsible for these phenomena (Supported by NIH ES07380 and EPA GOK20395).

1708 DOES AN ENVIRONMENTAL PCB/PCDF MIXTURE ENHANCE ULTRAVIOLET RADIATION-INDUCED SKIN CANCER?

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The incidence of non-melanoma skin cancer (NMSC) has been increasing significantly. Chemical carcinogens synergistically interact with solar ultraviolet radiation (UVR) in non-melanoma skin cancer development. PCBs/PCDFs are ubiquitous environmental contaminants and potential epithelial carcinogens that bioaccumulate in skin. Our objective was to examine the interaction between an environmentally relevant chemical mixture and UVR on skin carcinogenesis. Our aim was to test the hypothesis that living in a PCB/PCDF contaminated area would enhance UVR-induced skin carcinogenesis in the CRL:SKH-hrBr hairless mouse model of human NMSC. Four groups of mice were placed on either PCB/PCDF contaminated soil collected from an old electric landfill in Illinois or control soil for weeks 1-11. Mice were weighed weekly. Two weeks after the beginning of the experiment, 2/4 groups of mice, 1 on treated and 1 on control soil bedding, were exposed to UVR 5 days/week. Mice exposed to the chlorinated aromatic contaminated soil were significantly ($p<0.001$) heavier than those exposed to control soil. At week 18, papillomas were only present in the chemical mixture treated groups. Regardless of UV exposure, the tumor incidence and multiplicity were significantly ($p<0.001$) greater in the UVR/contaminated soil treated group compared with the no UVR/contaminated soil treated group. Based on the preliminary results, a synergistic interaction between contaminated soil and UVR on skin carcinogenesis is likely. Therefore, environmental exposure to chemical carcinogens could possibly be an important factor that enhances the incidence of NMSC.

1709 EFFECTS OF POLYCHLORINATED BIPHENYLS AND 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN ON PROLIFERATION AND CELL CYCLE REGULATION IN HUMAN PLACENTAL TROPHOBLASTIC JEG-3 CELLS.

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The environmental contaminants polychlorinated biphenyls (PCBs), dioxin and polyaromatic hydrocarbons have been linked to infertility and low birth weight in animals and humans. This study used the JEG-3 human placental trophoblastic cell line to investigate potential growth inhibitory effects of prototype organochlorines in relation to aryl hydrocarbon receptor (AhR) mediated induction of cytochrome P450 1A1. Cells were treated respectively with the AhR ligands 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), BENZO(A)PYRENE (B[a]P) and 3, 3', 4, 4'-tetraCB (TCB) in comparison with 2, 2', 4, 4', 5, 5'-hexaCB (HCB), which is not an AhR ligand. Following five-day treatment in the presence of 10% serum, [3H] thymidine incorporation was significantly decreased by 50 uM TCB, 50 uM HCB and 10 uM B[a]P, respectively, whereas 10 nM TCDD had no effect. Flow cytometry analysis of cell cycle phase distribution, however, showed no significant effects on the cell cycle distribution of cells treated with TCB, HCB or TCDD, whereas B[a]P treatment clearly produced a G2M arrest. Western immunoblot analysis showed no effect of TCB, HCB or TCDD on expression of the cell cycle inhibitory proteins p21 and p53. In contrast, B[a]P treatment strongly induced expression of p21 and, while total p53 levels were unchanged, serine-15 phosphorylation of p53 was markedly increased. The induction of CYP1A1 protein was seen only with TCDD and B[a]P, however, and not with either PCB congener. In conclusion, both HCB and TCB congeners significantly altered proliferation in the absence of cell cycle changes or CYP1A1 induction. In comparison, B[a]P-mediated growth inhibition was characterized by G2M cell cycle arrest and induction of p21WAF1 protein and p53 phosphorylation, which is a pattern reported for DNA damaging agents. Thus, data indicate that the TCB and HCB compounds decrease proliferation through an AhR-independent mechanism in JEG-3 cells. (Supported by NIH ES07375)

1711 CAUSATION ANALYSIS EXPOSES CRITICAL WEAKNESSES IN PURPORTED LINK BETWEEN PCBs AND NEURODEVELOPMENTAL DEFICITS.

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Causation analysis was applied to 24 epidemiology studies that sought an association between maternal PCB exposure and neurodevelopmental effects in 2, 573 children in 6 international cohorts. Our analysis judged: strength of association; consistency; evidence of a dose-response relationship; specificity of effect; biological plausibility; and temporality. Across the epidemiology studies, 5 analytical approaches were used to measure PCBs in 8 biologic media, resulting in more than 60 PCB metrics (including statistically generated composites) to assess exposure. Neurodevelopmental status was evaluated from birth to 11 years by means of 16 different tests assessing over 200 abilities. Of over 2, 200 comparisons between various exposure metrics and neurodevelopmental outcome measures, the number of statistically significant findings was fewer than expected by chance alone. All children were reported to be within normal ranges of performance. Performance of more highly PCB-exposed breast-fed children was not diminished, but was positively correlated with duration of breast-feeding. Some negative correlations reported between neurodevelopment and PCBs were equally attributable to lead or mercury. The findings were generally inconsistent and lacked specificity, credible evidence of a dose-response, or any assurance of a temporally correct relationship. Although the authors reported associations between PCB exposure and neurodevelopmental effects, our analysis found no support for that view. Those purported findings were more likely due to imprecise and inconsistent exposure measurements, poor measures of effect, uncorrected multiple comparisons and other improper statistical methods, and inadequate control for confounders and covariates. Thus, although there is biologic plausibility, a causal association between this ascribed cause (PCBs) and this purported effect (impaired neurodevelopment) is without empirical support.

1713 APPLICATION OF QSARs TO PREDICT TRANSFORMATIONS OF POLYHALOGENATED ALIPHATIC COMPOUNDS (PHAs) IN HOMOGENEOUS AND HETEROGENEOUS SYSTEMS.

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Under anaerobic reducing environments, PHAs may undergo reductive dehalogenation reactions, yielding products that may be less or more toxic than the parent compounds. The toxicity of the parent compounds and their dehalogenated products depend on their concentration in the environment, which in turn depends on

the kinetics of the dehalogenation reaction. Quantitative Structure Activity Relationships (QSARs) were developed to predict rates of transformation of PHAs in nine homogeneous and heterogeneous model systems obtained from literature. The model systems are representative of engineered and natural environments where PHAs are reduced either in the aqueous phase or at the surface of metals, oxides or hydroxides. The descriptors for predicting rates depended on the rate-limiting step in the compounds' reactions with reductants. Statistical techniques such as regression, factor and cluster analyses were used to examine rate-limiting steps, which are due to diffusion, sorption or dissociative electron-transfer (DET) in the transformation of PHAs in various studies. Descriptors that were chosen to describe DET were calculated using quantum chemistry, while diffusion and sorption of reactants and products to/from the surface were described using physical-chemical properties of the PHAs. Results from QSARs for homogeneous systems indicate that the rate-limiting step is dissociative one-electron transfer. Statistical analysis results indicate that in heterogeneous systems, the transformation of methanes is limited by electron transfer. The results also indicate that transformation of ethenes is limited by mass transfer, while ethanes are limited by both mass and electron transfer. Experiments were carried out for three new compounds to verify the QSARs for two different homogeneous systems. The difference between measured and predicted rate constants was within the uncertainty limits at 95% confidence, thereby demonstrating the predictive ability of QSARs for transformation of PHAs in homogeneous systems.

1714 PREVENTION BY CHLOROFORM AND METHIONINE OF DICHLOROACETIC ACID AND TRICHLOROACETIC ACID-INDUCED HYPOMETHYLATION OF GENES AND OF THE CHLOROACETIC ACIDS PROMOTION OF LIVER AND KIDNEY TUMORS IN MICE.

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Chloroform, dichloroacetic acid (DCA) and trichloroacetic acid (TCA) are mouse liver carcinogens. The effect of chloroform and methionine on DCA and TCA-induced hypomethylation of genes was determined. B6C3F1 mice were administered chloroform (0, 400, 800 and 1, 600 mg/l) in their drinking water and 500 mg/kg DCA or TCA daily by gavage. DCA, TCA and to a much lesser extent chloroform caused hypomethylation the c-myc and IGF-II genes. Co-administering chloroform prevented only DCA and not TCA-induced hypomethylation of the genes. Methionine at 0, 4, 000 or 8, 000 mg/kg diet was administered to B6C3F1 mice with 0, 3.5 gm/l DCA or 4.0 gm/l TCA administered in the drinking water. Methionine prevented both DCA and TCA-induced hypomethylation of the genes. The relationship of hypomethylation of genes to tumor development was tested in female and male B6C3F1 mice initiated on day 15 of age with N-methyl-N-nitrosourea. At 5 weeks of age, the mice started to receive in their drinking water DCA (3.2 gm/l) or TCA (4.0 gm/l). The mice also received chloroform at 0, 800 or 1, 600 mg/l in their drinking water or methionine at 0, 4, 000 and 8, 000 mg/kg in their diet. Chloroform prevented DCA, but not TCA promotion of liver foci and tumors. In male mice, TCA promoted kidney tumors while DCA promoted kidney tumors only when co-administered with chloroform. Hence, chloroform prevented only DCA and not TCA hypomethylation of the c-myc and IGF-II genes and promotion of liver tumors. Thus the ability to prevent hypomethylation of the genes was associated with the ability to prevent tumor promotion supporting the involvement of hypomethylation with tumor promotion.

1715 KINETIC AND MECHANISTIC STUDIES ON THE HYDROLYSIS OF HALOGENATED ETHYLENE OXIDES.

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Chlorinated ethylenes, e.g. trichloroethylene (TCE) and tetrachloroethylene (PCE) have been shown to be toxic and carcinogenic. P450-catalyzed biotransformation of these compounds to electrophilic ethylene oxides, followed by rearrangements of the oxides to acylating intermediates, are thought to be related to their toxicities. TCE oxide has been shown to generate adducts with lysine and proteins. In order to elucidate the inherent reactivity of these oxides, comparative kinetic studies on the hydrolysis and rearrangement were performed by using TCE oxide, tribromoethylene (TBE) oxide, and PCE oxide. These oxides decomposed in a MOPS/KOH buffer with first-order kinetics, TBE oxide being the most reactive. TCE and TBE oxides gave similar decomposed products of dichloroacetic acid (dibromoacetic acid in the case of TBE oxide), formic acid, glyoxylic acid and carbon monoxide, with different ratios. Dibromoacetic acid was the major product of TBE oxide, consistent with formation of a bromonium ion intermediate to give the acid through an intramolecular rearrangement. PCE oxide yielded trichloroacetic acid in a very low yield under the same conditions. In phosphate buffer, the oxide gave oxalyl phosphate and oxalic acid as well as trichloroacetic acid. These results indicate that PCE oxide decomposes through two pathways; one is an intramolecular chlorine migration to give trichloroacetic acid and the other is a hydrolysis of the oxide to give oxalyl chloride which is known to react to give the phosphate. The phosphate decomposed gradually to oxalic acid with first-order kinetics. Differences of reactivity in the decomposition reactions of these oxides may affect their reaction with intracellular nucleophilic molecules and both the pattern and extent of the adduct formation. (Supported in part by USPHS R01 ES10546 and P30 ES00267)

1716 MASS SPECTROMETRY STUDIES OF OLIGONUCLEOTIDES CROSS-LINKED TO ALKYLGUANINE TRANSFERASE *VIA* ETHYLENE DIBROMIDE.

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O^6-Alkylguanine transferase (AGT) is a DNA repair protein that normally removes alkyl groups from the O6 atom of guanine or the O4 atom of thymine, with Cys145 as the acceptor site for the alkyl group. However, AGT expression leads to increased cytotoxicity and mutagenicity of ethylene dibromide in *Escherichia coli* cells that lack endogenous AGT activity (Abril et al. *Mol. Carcinog.* **12**, 110-117, 1995; Liu et. al. *Mutat. Res.* **452**, 1-10, 2000). Ethylene dibromide or 2-bromoethanol was incubated with AGT in the presence or absence of oligonucleotides, and trypsin-digested samples were characterized by matrix-assisted laser desorption ionization (MALDI) and electrospray ionization mass spectrometry (MS). In the absence of oligonucleotides, both ethylene dibromide and 2-bromoethanol react with AGT to form an $-SCH_2CH_2OH$ adduct at Cys145; ethylene dibromide apparently forms this adduct *via* an episulfonium ion that reacts with water in the absence of other nucleophiles. When ethylene dibromide was added to AGT in the presence of a G-containing oligo and then subjected to trysin digestion, MALDI indicated that Cys145 was cross-linked to a G base. This result suggests that the episulfonium ion reacts with guanine at the N7 position, resulting in neutral thermal hydrolysis of the base during the trypsin digestion. Thus, the mutagenicity increase of ethylene dibromide in the presence of AGT may be due at least in part to the formation of abasic sites from guanine residues. Crosslinking of AGT to T was also observed with oligonucleotides. The contributions of the G and T modifications to mutation is under investigation. These findings provide an example of a DNA repair protein acting to increase the genotoxicity of a chemical. (Supported in past by USPHS R35 CA44353, R01 E10546, P30 ES00267, T32 ES07028)

1717 THE INFLUENCE OF ADVANCED AGE ON THE TIME COURSE OF THE HEPATIC TOXICITY OF BROMODICHLOROMETHANE.

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Chemical disinfection, by chlorination or by ozonation followed by either chlorine or chloramine, of water containing organic matter results in the production of a variety of halogenated byproducts, which includes trihalomethanes (THMs) such as bromodichloromethane (BDCM). Exposure to BDCM is virtually ubiquitous across all segments of the US population, including the aged. The present study evaluated the influence of advanced age on the time course of BDCM-induced hepatic toxicity. Young (~90 day old) and aged (22-24 months old) male F-344 rats were administered 0, 0.5, 1.0 or 2.0 mmol BDCM/kg by gavage in an aqueous vehicle (10% Alkamuls at a gavage volume of 5 ml/kg at ~ 3 p.m.). Hepatotoxicity was assessed, in separate experiments, at either 24 hr or 48 hr after dosing, by determination of the serum activities of sorbitol dehydrogenase (SDH) and alanine (ALT) and aspartate (AST) aminotransferases. The hepatotoxicity of BDCM was dose-dependent in both young and old rats. Based on these serum indicators, the hepatotoxic response to BDCM was dependent on age, with greater toxicity observed in the aged rats. At both 24 and 48 hr, the differences between young and aged rats were most apparent at the highest dosage (2.0 mmol/kg). At 2.0 mmol/kg, little or no recovery was apparent between 24 and 48 hr. These results are in contrast to results previously obtained (Lilly et al., 1997, McDonald et al, 2001) with chloroform, another THM ubiquitous in chlorinated or chloraminated water, where recovery was apparent in both young and aged rats between 24 and 48 hr and consistent with BDCM results in young rats (Lilly et al., 1997). In conclusion, these data suggest that advanced age alters the hepatic response to BDCM, indicating that the aged may be a susceptible subpopulation for THMs. (Abstract may not reflect EPA policy.)

1718 SENSITIVE METHODS FOR THE DETECTION OF TRACE LEVELS OF TRICHLOROETHYLENE (TCE) IN RAT PLASMA.

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TCE is a widely used solvent and metal degreasing agent that is frequently found in US drinking water supplies in trace levels (i.e. a few ppb to pptr). Current cancer and noncancer risk assessments of the chemical predict that exposure to even minute amounts of TCE may be associated with risks to humans. Due to a lack of analytical sensitivity, it has not yet been possible to conduct experiments to evaluate the validity of some key assumptions in the risk assessments. Two analytical methods were developed for this purpose and compared to one another. The first is a more traditional gas chromatography (GC) liquid-liquid extraction (LLE) method, while the second uses the relatively new technology of solid phase microextraction (SPME). With SPME, a fiber is inserted into the headspace of a sample vial to allow TCE to adsorb onto the fiber. The fiber is then placed into the injection liner of the GC where TCE is desorbed. SPME has the advantage of simpler, more rapid sample preparation. High resolution GC/MS was used for the analysis of plasma samples by both methods. For each technique, 100 uL of plasma were spiked with 100 uL of an aqueous TCE solution (1-10, 000 ng/mL). With LLE, 100-200 uL of diethyl ether were added to each sample. These samples were then centrifuged before analysis. Samples run by SPME were transferred to vials where the SPME fiber was inserted for 1 min, then removed and inserted into the GC for analysis. The limit of detection (LOD) was found to be 1 ng/mL (ppb) for both methods. This is an improvement over the LOD of 25 ng/mL for the GC headspace method currently in place in our laboratory. With the increased analytical sensitivity, trace level studies involving first-pass metabolism of TCE and interaction of TCE with inducers/inhibitors of metabolism are now possible. (Supported in part by DOE Grant # DE FC02 98CH10902 and ATSDR contract # 68164).

1719 PRESENCE AND ACTIVATION OF THE ARYL HYDROCARBON RECEPTOR IN NEURONAL VASCULAR ENDOTHELIUM.

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The aryl hydrocarbon receptor (AhR) is a member of the bHLH-PAS family of transcriptional regulatory proteins known functionally as a modulator of xenobiotic toxicity. Many of the mechanisms involved in AhR activation and the transcriptional events following ligand binding have been well characterized, but not all tissue-specific targets or functional consequences have been defined. Although the brain is a target for AhR mediated toxicity, consequences localized regions, such as the cerebral vascular endothelium are unknown. Receptor activation and subsequent CYP1A1 upregulation could compromise integrity and function of the neuronal endothelium and therefore, the blood-brain barrier. The purpose of these studies was to examine the presence and activity of AhR in brain endothelium. Receptor mRNA and protein were found regionally throughout the brain of adult C57BL/6 mice. Relative quantitation by real-time RT-PCR indicated receptor mRNA within the cerebellum > cortex and stratum > thalamus and hypothalamus. Furthermore, the receptor was also detected in total RNA and protein isolated from primary cortical endothelial cultures. Although its abundance is nearly ten-fold less in the whole cortex and endothelial cell culture than in liver, exposure to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), induced upregulation of CYP1A1 transcription. We hypothesize that receptor activation and downstream gene transcriptional upregulation in these cells may have functional consequences in terms of oxidative stress and barrier permeability. If these consequences occur *in vivo*, they could play a significant role in the potentiation of vascular injury that may underlie the development of various neurodegenerative diseases. (Supported by NIH grant ES09430, Training Grant ES07026, and Center Grant ES01247)

1720 KERATINOCYTE INTERLEUKIN-1β EXPRESSION IS MEDIATED BY THE ARYL HYDROCARBON RECEPTOR.

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Halogenated aromatic hydrocarbons represent a wide variety of environmental contaminants. Human exposure to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most potent member of the halogenated aromatic hydrocarbon family, produces several adverse health effects, including chloracne, a skin rash characterized by epidermal hyperkeratinization and inflammation. The proinflammatory cytokine interleukin-1β (IL-1β) has previously been suggested to play a role in the pathology of chloracne. TCDD elicits its effects by binding to the aryl hydrocarbon receptor (AhR) and forming an AhR heterodimeric nuclear complex. To test the hypothesis that the IL-1β gene is regulated by an AhR-mediated mechanism, differentiating SCC-12 and HaCaT human keratinocytes were treated with TCDD or vehicle. Western blot analyses were performed to determine the effect of TCDD on IL-1β protein expression in these keratinocytes. Time and dose dependent increases in prointerleukin-1β (pIL-1β) were seen in both SCC-12 and HaCaT cells following TCDD treatment when compared to vehicle controls. Northern blot analyses were performed to ascertain the effect of TCDD on IL-1β steady state mRNA levels. TCDD treatment resulted in dose and time dependent increases in IL-1β mRNA in both SCC-12 and HaCaT cells when compared to vehicle controls. Increases in CYP1A1 mRNA, a gene known to be transcriptionally activated by TCDD in cells undergoing terminal differentiation, paralleled increases in IL-1β mRNA with respect to time in both cell lines. Both cell lines were treated with TCDD in the presence and absence of α-naphthoflavone, an AhR antagonist, to determine if the TCDD-mediated increases in IL-1βprotein and steady state mRNA were AhR-dependent. Western blot and Northern blot analyses revealed that the AhR antagonist attenuated TCDD-mediated increases in IL-1β. These results confirm that the AhR regulates TCDD-mediated increases in IL-1β gene expression in differentiating keratinocytes. (Supported, in part, by NIH ES05968).

1721 DIOXIN TOXICITY: LARGE-SCALE GENE EXPRESSION IN A RESISTANT RAT MODEL.

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2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is exceptionally toxic and also alters expression of a wide spectrum of genes in cell culture and *in vivo via* the AH receptor (AHR). Dysregulated gene expression probably triggers toxicity but the dioxin-regulated genes that are key to lethality and other toxicities remain ill-defined. We are using gene expression arrays to identify and to compare TCDD-responsive genes in rat strains that differ dramatically in their sensitivity to TCDD lethality and in their AHR structure: Long Evans *Turku AB* (LE) [LD_{50} ~ 10 μg/kg] and Han/Wistar *Kuopio* (H/W) [LD_{50} > 9600 μg/kg]. Our postulate is that genes whose expression is altered by TCDD in sensitive LE rats but not in resistant H/W rats are the genes most likely to be relevant to lethality. Rats were given a single TCDD dose of 50 μg/kg intragastrically. Liver was removed after 24 hours and RNA was reverse-transcribed and labeled with ^{32}P prior to hybridization to Clontech Atlas 1.2 rat arrays. Of 1185 genes on the array, TCDD caused 6% (67 genes) to change in expression (1.5-fold, up or down) in the LE rat and 8% (90 genes) in the H/W. Of these, only 33 were genes common to both strains. Genes well-established as being responsive to dioxins (*CYP1A1, CYP1A2, CYP1B1, GST-Ya*) were upregulated in both LE and H/W strains, establishing the validity of this array for detection of responses to TCDD. In addition to altered expression of these "positive control" genes, the experiments revealed altered expression of several novel genes not previously reported to be affected by TCDD. Additional rat strains & lines now are being studied with the Clontech array and with a glass microarray from the Ontario Cancer Institute (>15, 000 unique cDNA sequences) to establish a hierarchy of genes whose TCDD-responsiveness consistently differs between dioxin-sensitive *versus* dioxin-resistant rats to identify candidate genes that are most likely to be involved in dioxin lethality.

1722 LOW *IN VITRO* HEMATOPOIETIC TOXICITY OF TENOFOVIR RELATIVE TO OTHER NRTIS: COMPARISON OF RAPID THROUGHPUT CELISA AND COLONY ASSAY DATA.

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Nucleoside reverse transcriptase inhibitor (NRTI)-containing regimens have been demonstrated to produce various hematological disorders. In comparison with untreated control groups, anemia and/or neutropenia have been detected at a higher rate in HIV patients treated with ZDV (Richman et al., 1987), d4T (Browne et al., 1993), or ddC (Yarchoan et al., 1988). Tenofovir is an acyclic nucleotide analog with potent *in vitro* and *in vivo* antiretroviral activity. Its lipophilic prodrug, tenofovir disoproxil fumarate, is currently in late stage clinical development as a novel oral agent for treatment of HIV infection. The present study was designed to help define the *in vitro* toxicity profile of tenofovir and other NRTIs by assessing their cytotoxic effects in human hematopoietic progenitor cells. Two different *in vitro* toxicity assays were used: the clonogenic "colony assay" and the 96-well filter plate-based CELISA. Human $CD34^+$ progenitor cells were isolated from two individual

donors, treated with lineage-specific growth factors to induce myeloid and erythroid differentiation and exposed to NRTIs for 10 days. Lineage-specific differentiation was determined by use of the CELISA platform technology utilizing lineage-specific fluorescent antibody-based immunoassays and colony counting assays. The tested NRTIs displayed a range of toxicities with IC_{50} values ranging from >200μM to <60nM. The relative toxicities of the NRTIs were ddC > ZDV > d4T > 3TC ≅ tenofovir. ZDV and d4T had a >10-fold increased myelotoxicity in one donor relative to the second and exhibited marked lineage-specific toxicities, the erythroid lineage being the more sensitive in each case. The CELISA data correlated with colony assay data in all cases. We conclude that lead optimization of NRTIs and other drugs can be greatly accelerated through use of the rapid-throughput CELISA assay of *in vitro* cell differentiation.

1723 THE ROLE OF REACTIVE OXYGEN SPECIES IN MEDIATING THE ACTIVITY OF THE C-MYB ONCOGENE.

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The incidence of childhood leukemias has increased 20% over the last two decades. Exposure to several drugs and environmental toxicants has been proposed to be a primary cause for this increase. Mutated or overexpressed versions of the *c-myb* oncogene are believed to play a pivotal role in the development of a variety of leukemias and other tumours. The *c-myb* gene encodes a highly conserved 78 kDa transcription factor that has been implicated in a diverse set of biological processes, including the regulation of cell proliferation, differentiation and apoptosis. While the structure of the c-Myb protein and the regulation of *c-myb* gene expression have been studied in great detail, little is known about the upstream activators that regulate c-Myb protein activity. Furthermore, nothing is known about the effects of xenobiotic exposure on c-Myb activity. The toxicity of many xenobiotics is mediated by xenobiotic bioactivation to a reactive free radical intermediate, which if not detoxified may initiate the formation of reactive oxygen species (ROS). We hypothesize that exposure to xenobiotics that lead to increased ROS mediate leukemogenesis by disrupting crucial cell signalling pathways involving the *c-myb* oncogene. To evaluate this hypothesis, quail QT6 cells were transfected with a Myb-responsive luciferase reporter plasmid derived from the chicken *mim-1* promoter, along with control vector DNA or a plasmid expressing c-Myb. The following day cells were serum starved for 24 hr and then exposed to 400 μM H_2O_2 for 5 minutes. Luciferase activities were then measured in cell lysates to evaluate c-Myb activity. Results of these studies showed that cells exposed to H_2O_2 had significantly increased c-Myb activity compared to cells exposed to the vehicle control and support the hypothesis that ROS mediate the transcriptional activity of this oncogene. The hypothesis that xenobiotic-induced ROS production leads to aberrant c-Myb signalling is currently being investigated.

1724 SIMULTANEOUS DETERMINATION OF SIX URINARY PORPHYRINS IN THE UNTREATED BEAGLE DOG USING LIQUID CHROMATOGRAPHY - TANDEM MASS SPECTROMETRY.

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Porphyrin is converted to heme through a series of enzymatic steps. Deficiencies in these enzymes can lead to porphyria, which can have specific toxicological consequences. Evaluation of porphyrins in urine can be useful in the recognition of these toxicological sequelae. A liquid chromatographic-tandem mass spectrometric (HPLC-MS-MS) method without sample pretreatment was developed and validated for determination of urinary porphyrins. The acidified urine sample was directly injected for HPLC/MS/MS analysis using a reverse-phase column with gradient elution, and ESI positive ion detection and quantitation. The mass spectrometer was operated in the multi-reaction monitoring (MRM) mode and six porphyrins (mesoporphyrin, coproporphyrin, 5-carboxyporphyrin, 6-carboxyporphyrin, 7-carboxyporphyrin and uroporphyrin) were detected with excellent sensitivity and selectivity. Values obtained in the untreated beagle dog corresponded to not present, 0.1361, 0.0196, below lower limit of quantification, 0.0328 and 0.0624 nmol/mL respectively, which are within expected normal values. The lower limit of quantitation was 0.014 nmol/mL for each porphyrin, and a good linear response for calibration standards ranging from 0.01 to 1.0 nmol/mL for each porphyrin was demonstrated. This method can be used for determining the effects of chemicals and pharmaceuticals on urinary porphyrin excretion profiles in preclinical animal studies and in the investigation or diagnosis of porphyria in clinical studies.

1725 *IN VIVO* DISTRIBUTION AND TOXICITY OF THE NOVEL COPPER-BASED ANTICANCER COMPOUND: CASIOPEINA II.

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The *in vivo* fate and toxicity of the novel copper-based anticancer agent, casiopeina II [Cu(4, 7-dimethyl-1, 10-phenanthroline)(glycine) NO_3], was studied. Casiopeinas are a family of square planar copper-coordinated complexes that have shown promising anticancer activity. The phenanthroline ligand of CII was tritium labeled to follow the distribution of the two components of the complex. After a single iv administration of ^{3}H-casiopeina II (CII) (5mg/kg dose) in the rat the complex was rapidly distributed to tissues (liver and kidney), the ^{3}H-phenathroline was excreted *via* urine and feces whilst the copper appeared to be predominantly retained with a small amount being excreted *via* feces. Differences in tissue copper retention and ^{3}H-phenanthroline elimination suggest an *in vivo* dissociation of the complex. The major toxic effect attributed to CII administration was an hemolytic anemia (reduced hemoglobin concentration, red blood cell count and packed cell volume as well as leukocytosis and neutrophilia) observed 12 hours and 5 days after administration, attributed to a direct erythrocyte oxidative damage. Increased reticulocyte levels and presence of normoblasts in peripheral blood 5 days post-administration indicated an effective erythropoietic response and tendency towards recovery at 15 days. Increase in spleen weight was attributed to an increased uptake of damaged erythrocytes by the reticuloendothelial system. Erytropoietin pretreatment before CII administration reduced the hematological effects. In conclusion data from copper and ^{3}H distribution and excretion suggests that CII complex is readily dissociated *in vivo*, which could give rise to the acute toxic effect in erythrocytes due to the generation of free radicals and oxidative stress from the reduction of Cu(II) to Cu(I). Improving the stability of the complex *in vivo* could increase its biological activity and thus its anticancer potential.

1726 SINUSOIDAL ENDOTHELIAL CELL INJURY DURING HEPATOTOXICITY FROM MONOCROTALINE AND BACTERIAL LIPOPOLYSACCHARIDE COEXPOSURE.

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A previous study demonstrated that a nontoxic dose of bacterial lipopolysaccharide (LPS) given 4 hours after a small dose of monocrotaline (MCT) resulted in synergistic hepatotoxicity within 12 to 18 hours after MCT administration. Histopathologic analysis revealed centrilobular and midzonal lesions characteristic of those associated with larger toxic doses of MCT and LPS, respectively. Moreover, within these lesions there was pronounced hemorrhage, loss of sinusoidal architecture, and the loss of central vein intima, suggesting a vascular component to the injury. It has been postulated that injury to sinusoidal endothelial cells (SECs) creates microcirculatory disturbances that contribute to hepatic parenchymal cell injury. To begin to explore this hypothesis, the association of SEC injury with hepatic parenchymal cell injury was evaluated. At 18 hours, a significant increase in plasma hyaluronic acid (HA), a biomarker of SEC injury, was observed in animals cotreated with MCT/LPS. This increase in SEC injury was confirmed through immunohistochemistry with a rat endothelial cell antibody (RECA-1). Morphometric analysis revealed a decrease in RECA-1 staining intensity (i.e., a loss of SECs) in livers of rats treated with MCT/LPS. The decreased staining occurred in both centrilobular and midzonal regions of the liver lobule. A significant increase in liver hemoglobin, a biomarker of hemorrhage, was also observed at 18 hours in cotreated animals, further suggesting circulatory and vascular disturbances. Finally, SEC injury developed before the onset of hemorrhage and parenchymal cell injury, an observation consistent with the hypothesis that SEC injury plays a causal role in this model of liver injury. *Supported by NIH Grant ES 04139.*

1727 NAPROXEN SODIUM NEPHROTOXICITY: INTERSPECIES DIFFERENCES.

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Cyclooxygenase (COX) exists in two isoforms, COX-1 and COX-2. Constitutive COX-2 is found in the macula densa (MD) and thick ascending limb of the loop of Henle in rodents and dogs, and is absent at these sites in normal adult humans and monkeys. This difference in COX expression may be responsible for the contrasting nephrotoxicity profiles of NSAIDs in these species. We compared the toxicity profile of naproxen sodium (a non-selective COX inhibitor) in dogs and monkeys.

Beagle dogs and cynomolgus monkeys were given naproxen sodium orally for six weeks at dosages up to 10 and 150 mg/kg/day. The dogs exhibited a 5-fold increase in BUN and a 1.1-fold increase in creatinine suggesting pre-renal azotemia. Kidneys exhibited tubular atrophy and fibrosis in the outer cortex of 4/6 dogs and renal papillary necrosis in 3/6 dogs. The lesions correlated with increases in BUN. In contrast, monkeys exhibited a less pronounced increase in BUN (2.7-fold) and did not develop renal papillary necrosis or cortical tubular atrophy and fibrosis; however, obstructive nephropathy, presumably due to drug accumulation in the renal tubules, was observed. Monkeys also exhibited an increase in creatinine (3.6-fold) which was likely secondary to the obstructive nephropathy. These data suggest that dogs are more sensitive to NSAID-related nephrotoxicity in comparison to monkeys. The renal changes in dogs appear to be secondary to regional hypoxia/ischemia and implicate an effect of naproxen sodium on renal hemodynamics. The MD plays an important role in renin angiotensin system; thus, co-localization of high levels of COX-2 at this site in dogs suggest an important role of COX-2-mediated prostaglandins in normal renal physiology in dogs. The role of COX-2 on renal hemodynamics in the normal monkey appears to be less significant. This study demonstrates that a differential renal response to NSAID administration among species can be explained by the interspecies variation of renal COX-2 expression and its species specific role in normal kidney function.

1728 NEUTROPHIL APOPTOSIS AND THE RESOLUTION OF PULMONARY INFLAMMATION.

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The apoptosis, or programmed cell death, of neutrophils and their subsequent engulfment by macrophages is important in the resolution of pulmonary inflammation. Previous studies showed a correlation between inflammation and apoptosis in silica-exposed rats. However, in titanium dioxide (TiO2) exposed rats, only a slight apoptotic potential was seen at very high doses even in the presence of measurable levels of inflammation. The goal of the current study is to further investigate the mechanism of apoptosis and its correlation to the inflammatory process due to amorphous and crystalline silica, LPS and TiO2 exposure. Rats were instilled with TiO2, Zeofree 80 (amorphous) or Min-U-Sil (crystalline silica) at both 1 and 5 mg/kg. A group of rats were also instilled with 6 mg LPS. Bronchoalevolar lavage fluid and tissue were collected at 12, 24, 48 and 168 hours after instillation. Only the amorphous silica instilled animals displayed measurable apoptosis (detected by ELISA) at 1 mg/kg with the highest level at 24 hours post instillation. At 5mg/kg, apoptosis was highest at 12 hours for amorphous silica, and at 24 hours for the other substances tested. By 168 hours, only crystalline silica instilled animals had any measurable response. Pulmonary inflammation, as evidenced by neutrophil influx correlated with the apoptotic measurements. cDNA array technology was employed on lung tissue from sham, Zeofree 80, Min-U-Sil and LPS animals and expression of several genes related to apoptosis were upregulated. These studies confirmed that TiO2 exposure causes low levels of inflammation and apoptosis. Amorphous silica and LPS produce a strong, but transient inflammatory response while instillation of crystalline silica results in sustained pulmonary inflammation and measurable apoptosis after a week.

1729 COMPARISON OF LOW DOSES OF AGED AND FRESHLY FRACTURED SILICA ON PULMONARY INFLAMMATION AND DAMAGE IN THE RAT.

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Most previous studies of silica toxicity have used relatively high doses of silica. In this study, rats received by intratracheal instillation either vehicle, aged or freshly fractured silica at a dose of either 5 ug per rat once a week for 12 weeks (total dose of 60 ug) or 20 ug per rat once a week for 12 weeks (total dose of 240 ug). One week after the last exposure, bronchoalveolar lavage (BAL) was conducted and pulmonary inflammation, alveolar macrophage (AM) activation and pulmonary damage were examined. Specifically, pulmonary inflammation was assessed by BAL polymorphonuclear leukocyte (PMN) yields, AM chemiluminescence was measured as an indicator of AM activation, and first acellular BAL fluid lactate dehydrogenase activity and serum albumin concentration were determined as markers of pulmonary damage. For rats exposed to a total of 60 ug silica, both aged and freshly fractured silica increased PMN yield and AM chemiluminescence above control to a similar degree, but no evidence of pulmonary damage was detected. For rats exposed to 240 ug silica, aged or freshly fractured silica increased PMN yield and AM chemiluminescence above control. However, total and NO-dependent AM chemiluminescence was greater for rats exposed to freshly fractured silica compared to aged silica. Exposure to 240 ug aged or freshly fractured silica also resulted in pulmonary damage, but the extent of this damage did not differ between the two types of silica. The results suggest that exposure to silica levels far lower than those previously examined can cause pulmonary inflammation and damage. In addition, exposure to freshly fractured silica causes greater generation of reactive oxygen and nitrogen species from AMs (measured as total and NO-dependent AM chemiluminescence) in comparison to aged silica, but there is an apparent threshold below which this difference does not occur.

1730 INCREASED IL-6 AND IL-8 EXPRESSION IN HUMAN AIRWAY EPITHELIAL CELLS EXPOSED TO CARBON ULTRAFINE PARTICLES.

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Ultrafine particles are a potentially toxic constituent of particulate matter (PM) and may contribute to the health effects of ambient PM. Exposure to extracts or components of PM has been shown to evoke inflammatory responses in cultured normal human airway epithelial cells (NHBE). One of these responses is the expression of pro-inflammatory proteins such as IL-6 and IL-8. The aim of the present study was to characterize the effect of ultrafine elemental carbon particle (EC) exposure on IL-6 and IL-8 expression in NHBE. Particles were generated by spark discharge and had a diameter of 75 nm and a surface area of 750 m^2/g. Cells were exposed to 0 to 66 ug/ml of EC for 2 to 30 hr and levels of IL-6 and IL-8 mRNA and protein were measured using real-time fluorescent PCR and ELISA, respectively. After 2 hr of exposure to 33 or 66 ug/ml EC, there were minimal increases in IL-6 and IL-8 mRNA when compared to untreated controls. However, a 24 hr exposure to 33 ug/ml EC showed 9-fold and 12-fold increases in IL-6 and IL-8 mRNA, respectively. Doubling the amount of EC to 66 ug/ml resulted in a 10-fold increase in IL-6 mRNA and a 20-fold increase in IL-8 mRNA. To minimize underestimation of IL-6 and IL-8 protein release caused by non-specific protein binding to EC, a pulsed exposure protocol was used. After exposing NHBE to media with 33 or 66 ug/ml EC for 8 hr to initiate mRNA induction, media was changed so that protein release would take place in media without EC. When compared to controls, this regimen resulted in 5-fold elevations in IL-6 and IL-8 release by NHBE that were exposed to EC for 24 hr. These data show that carbon ultrafine particle exposure induces inflammatory mediator expression, suggesting a contributing mechanism for the health effects of ambient PM inhalation. Further studies will be required to identify the mechanism of IL-8 expression induced by exposure to EC. **This abstract of a proposed presentation does not necessarily reflect EPA policy.**

1731 RESPONSIVENESS OF ALVEOLAR MACROPHAGES FROM INOS KNOCKOUT OR WILD TYPE MICE TO AN *IN VITRO* LPS OR SILICA EXPOSURE.

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The role of nitric oxide (NO) in pulmonary disease has been controversial with both anti-inflammatory (scavenging radicals and inhibiting NF-κB activation) and pro-inflammatory (forming highly reactive peroxynitrite and augmenting NF-κB activation by inflammatory agents) actions reported. Therefore, a project has been initiated to determine whether deletion of the iNOS gene in the C57BL/6J mouse alters the pulmonary response to lipopolysaccharide (LPS) or silica. The objective of the initial phase of this study was to determine the responsiveness of alveolar macrophages (AM's), harvested from naive wild type (WT) or iNOS knockout (KO) mice, to an *in vitro* LPS or silica exposure. Primary AM's were obtained by bronchoalveolar lavage from age and weight matched iNOS KO and WT mice. The cells were treated with IFN-γ (50 U/ml), IFN-γ (50 U/ml) plus LPS (1μg/ml), LPS (0.01 -100 μg/ml), or silica (25 - 250 μg/ml). Inflammatory parameters included: NO, tumor necrosis factor-α (TNF-α) and macrophage inflammatory protein-2 (MIP-2) production, and intracellular generation of hydrogen peroxide and superoxide. Data show a significant increase in NO production upon exposure to IFN-γ +/- LPS in the WT but not iNOS KO AM's. NO production by KO or WT AM's was not increased by *in vitro* exposure to LPS or silica alone. LPS, but not silica, induced TNF-α and MIP-2 production with the KO AM's being more responsive than WT. Basal intracellular production of hydrogen peroxide and superoxide was significantly greater in KO compared to WT AM's. In addition, LPS (10 μg/ml) or silica (100 μg/ml) stimulated intracellular oxidant production was lower in KO AM's. In conclusion, when compared to WT AM's, iNOS KO AM's exhibit a decreased ability to generate reactive oxygen species in response to LPS or silica. However, iNOS KO AM's exhibit enhanced inflammatory cytokine and chemokine production in response to LPS.

1732 EFFECTS OF OIL COMBUSTION EMISSION EXPOSURE ON ACUTE LUNG INJURY AND SUSCEPTIBILITY TO INFECTION IN MICE.

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It has been suggested that the increased morbidity and mortality associated with exposure to airborne particulates (PM) is related to materials derived from combustion sources such as gasoline, diesel, oil, wood and coal. The purpose of this project

was to develop a system that allows for the generation of primary combustion particles followed by controlled dilution and conditioning, and subsequent whole-animal inhalation exposure. In these pilot studies a heavy oil # 5 was burned in a residual oil combustor previously shown to produce approximately 150 mg/m3 of PM. The particles were then passed through a 2.2 μM cyclone and diluted with clean ambient air. The resulting PM was then directed to a Hinners animal exposure chamber equipped for continuous monitoring of O_2, Co., CO_2, NO_x, SO_2, as well as total particle concentrations. Mean PM 2.5 concentrations ranged between 2 and 3 mg/m-3 with 40-50 ppm SO_2 and 20-30 ppm NO_x. Balb/C and CD1 mice were exposed to the combustion atmosphere for 4 hrs and immediately and 24 hr later assessed for acute lung injury (both strains) and susceptibility to Streptococcus zooepidemicus infection (CD1 strain). Exposure to the emission atmosphere caused a significant increase in neutrophils and lactate dehydrogenase (LDH) in bronchoalveolar lavage (BAL) of both strains of mice but did not affect levels of protein. Mortality to infection was low in all animals and was not affected by the emission exposure. We conclude that this acute exposure caused mild pulmonary injury and inflammation in a similar fashion to other oxidant pollutants but did not affect host defenses to streptococcal infection. Future studies will determine the relative contribution of gases *versus* particles on these inflammatory responses, and will test whether animals with other forms of cardio-pulmonary disease are further compromised by such exposures. This abstract does not necessarily reflect EPA policy.

1733 COMPARISON OF SINGLE AND MULTIPLE EXPOSURES OF CONCENTRATED AIR PARTICLE (CAPS)ON PATHOPHYSIOLOGIC RESPONSES IN HEALTHY RATS.

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Several studies have reported that exposure to CAPS from various cities causes pulmonary inflammation in rats. The purpose of this study was to determine whether CAPS from the Research Triangle Park area could cause similar effects, and to compare responses between single and multiple CAPS exposures. Using a 4 stage Harvard concentrator system which concentrated particles 80-fold, male Sprague-Dawley rats were exposed to CAPS for 4 hours, 3 times a week for 5 weeks. During the last 2 exposures (chamber concentrations of 0.57 and 1.7 mg/m3 respectively), additional groups of animals were exposed to CAPS or clean air and their acute pulmonary inflammatory responses (18 hr post-exposure)were compared to animals which had been exposed for the entire 5 week period. After 2 and 4 weeks of exposure, animals monitored with a Buxco plethysmograph system showed a small exposure-related decline in enhanced pause values, suggesting an alteration in ventilatory function. At necropsy, no changes were apparent in bronchoalveolar lavage levels of protein and LDH. The higher CAPS concentration however, caused an increase in pulmonary alveolar macrophage and neutrophils. This effect seemed to be driven by the last exposure as animals exposed 14 times to CAPS prior to this exposure did not have significantly greater responses. Further, exposure to the lower concentration had no effect indicating either; a Dose-Response effect, or changes in the physico-chemical makeup of the PM over the last 2 exposures. Other significant differences included a decrease in lung ascorbate levels following acute exposure to the higher CAPS concentration, and an acute, transient increase in plasma fibrinogen levels. The data show that acute CAPS exposure causes a dose-dependent pulmonary infiltration of cells, depletion of anti-oxidants, and increased potential for systemic coagulation. These effects did not appear to be worsened with multiple exposures. This abstract does not reflect EPA policy

1734 PARTICULATE 1->3-β-GLUCAN IS THE MORE POTENT FORM FOR INDUCING PULMONARY INFLAMMATION IN RATS.

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1->3-β-Glucans, derived from the inner cell wall of yeasts and fungi, are commonly found in indoor air dust samples and have been implicated in organic dust toxic syndrome. In a previous study, we reported that 1->3-β-glucan (zymosan A) induced acute pulmonary inflammation in rats. The present study investigates which form of 1->3-β-glucans, particulate or soluble, is more potent in inducing pulmonary inflammation. Zymosan A was suspended with 0.25 N NaOH for 30 min, neutralized, dialyzed for 2 days against deionized water, and particulate and soluble fractions collected. Male Sprague-Dawley rats were exposed *via* intratracheal instillation to NaOH-soluble or NaOH-insoluble zymosan A (1.8 mg/ml, 0.26 ml). At 18 hrs post-exposure, various indicators of pulmonary response were monitored, including indicators of lung damage, such as serum albumin concentration and lactate dehydrogenase activity in acellular bronchoalveolar lavage fluid. Inflammation was characterized by an increase in lavageable polymorphonuclear leukocytes (PMN), pulmonary irritation (breathing frequency increase) and oxidant production (nitric oxide and chemiluminescence (CL)). Exposure to the particulate fraction of NaOH-treated zymosan caused a significant increase in all these indicators. In contrast, rats exposed to the NaOH-soluble fraction did not show significant increase for most of these indicators except for albumin, PMN and CL. However, these increases were significantly smaller than with exposure to NaOH-insoluble zymosan. Therefore, the results demonstrate that particulate zymosan A is more potent in inducing pulmonary inflammation and damage in rats than the soluble form of this β-glucan.

1735 VANADIUM-INDUCED PULMONARY INFLAMMATION AND APOPTOSIS IN MICE.

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Pulmonary exposure to vanadium and vanadium-containing compounds is associated with acute pulmonary inflammation, characterized by a rapid influx of polymorphonuclear neutrophils with a peak response at 6 h and resolution by 3 d. Sodium metavanadate [V (V)] can induce cell apoptosis *in vitro*, but little is known about relationship of V (V) and lung cell apoptosis *in vivo*. We hypothesized that vanadium may induce lung cell apoptosis through reactive oxygen species (ROS) *in vivo* and neutrophil apoptosis is involved in the resolution of vanadium-induced lung inflammation. To test this hypothesis, mice were treated with V (V) or saline control, and the bronchoalveolar lavage (BAL) cells were examined at various times for short-lived free radicals by electron spin resonance (ESR) and for apoptosis using terminal deoxyribonucleotidyl transferase-mediated nick end labeling (TUNEL). Control mouse BAL cells showed no significant free radical activity and only resident alveolar macrophages in the BAL fluids with no evidence of apoptosis. In contrast, V (V) induced a greater level of free radicals and caused apoptosis of BAL cells which are predominantly neutrophils. Catalase blocked both free radical generation and apoptosis induced by V (V). The number of apoptotic cells gradually increased and reached a maximal level by 24 h where it subsequently declined. After 24 h when the vanadium-induced lung inflammation was in the resolution phase, we observed an increased influx of macrophages and their engulfment of apoptotic bodies in the BAL fluid. At 72 h when the total number of neutrophils fell to the baseline level, remnants of apoptotic bodies could still be seen in the cytoplasm of macrophages. We conclude that (1): ROS, like H_2O_2, are one of causes of apoptosis induced by vanadium; (2): apoptosis of neutrophils and clearance by macrophages is an important mechanism in the resolution of vanadium-induced lung inflammation.

1736 SYMPATHETIC NERVOUS SYSTEM PLAYS A MAJOR ROLE IN ACUTE COLD/RESTRAINT STRESS INHIBITION OF HOST RESISTANCE TO *LISTERIA MONOCYTOGENES*.

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Multiple studies have demonstrated that both glucocorticoids and norepinephrine (NE) can modulate immune responses *in vitro* and *in vivo*, and it is well known that the sympathetic nervous system innervates both primary and secondary lymphoid organs. Here, we show that acute cold/restraint stress (ACRS) significantly lowers host resistance to *Listeria monocytogenes* (*LM*) in BALB/c mice, and the involvement of stress hormones corticosterone (CORT) and NE is evaluated. CORT and NE were investigated by pretreating mice with the CORT synthesis inhibitor metyrapone and the chemical sympathectomy drug 6-hydroxydopamine (6-OHDA), respectively. *LM* burdens were determined 3 days post-infection. 6-OHDA significantly decreased the *LM* burden in both control and stressed animals. 6-OHDA also completely blocked the stress effects observed in spleens while only partially affecting the liver. The 6-OHDA-uptake inhibitor desipramine confirmed that peripheral sympathetic adrenergic nerves and NE depletion and not the nonspecific 6-OHDA toxicity were responsible for the enhanced host defense. In contrast, metyrapone-treated animals had further decreased host resistance to *LM* after ACRS. The results suggest that the peripheral sympathetic nervous system (SNS) postganglionic neurotransmitter NE plays a major role in *LM* host resistance, but there are significant tissue-dependent effects after ACRS, while CORT provides a potential protective effect after ACRS. Altogether, stress hormones play important roles in stress-modulated host resistance and NE is a major hormone involved in ACRS-induced suppression of host resistance in the spleen but not the liver. (Supported by NYSDOH and NIEHS ES15-3506A).

1737 *ASPERGILLUS VERSICOLOR* ISOLATED FROM INDOOR AIR OF A MOISTURE-DAMAGED BUILDING PROVOKED ACUTE INFLAMMATION IN MOUSE LUNGS.

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Sponsor: M. Viluksela.

Moisture related microbial growth in buildings is associated with respiratory symptoms and inflammatory reactions. *Aspergillus versicolor* is a mycotoxin producing fungus detected in the air and materials of water-damaged buildings. We investigated the dose-response and time course of the effects induced by the spores of *A. versicolor*, isolated originally from indoor air of a moisture-damaged building, after a single intratracheal dose instilled into NIH/S mouse lungs. In dose-response study ($1x10^5$, $1x10^6$, $5x10^6$, $1x10^7$ and $5x10^7$ spores) lungs were lavaged at 24 hours, and in time course study ($5x10^6$ spores) at time points 6 h, 24 h, 3 d, 7 d, 14 d, 21 d and 28 d. The concentrations of the cytokines (tumor necrosis factor alpha (TNFα) and interleukin (IL)-6) in bronchoalveolar lavage fluid (BALF) were measured by ELISA method, total cell number was counted by the Trypan blue exclusion method, and cell differentials were determined from cytospin plates. The spores caused dose- and time dependent increase in both TNFα and IL-6 concentrations. Both cytokines peaked at 6 h, and the responses were over by 3 days. The number of neutrophils peaked at 24 h, and practically leveled off by 3 days. Macrophages peaked at 3 and lymphocytes at 7 days. Lymphocyte response was transient, but macrophages remained at a slightly higher level until the end of the study. Our results suggest that *A. versicolor*, isolated from a moldy building, is able to cause an acute inflammation in mouse lungs. (Supported by the SYTTY Programme, Academy of Finland.)

1738 CYCLOOXYGENASE-2 INHIBITION DOES NOT AFFECT MONONUCLEAR PHAGOCYTE FUNCTION.

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Two isoforms of cyclooxygenase (COX), COX-1 and COX-2, catalyze the production of prostaglandins (PGs) involved in various physiologic and pathophysiologic processes. COX-2 is the only isoform induced at the site of inflammation and is responsible for the generation of pro-inflammatory PGs. It is not known whether inhibition of COX-2 at the site of inflammation will affect primary inflammatory cell functions. Therefore, a series of *in vitro* and *in vivo* studies were conducted to evaluate the functional capacity of mononuclear phagocytes following treatment with a selective COX-2 inhibitor (SC-58236) and a non-selective COX inhibitor (naproxen sodium). The *in vitro* studies evaluated the effects of these drugs (1, 10, or 100μM) on the capacity of rat peritoneal macrophages to phagocytose opsonized *S. aureus* coated bioparticles. Cytochalasin B, a microfilament disruptor, was used as a positive control. The *in vivo* study evaluated the functional activity of tissue-specific macrophages by monitoring the clearance of ^{51}Cr-labeled sheep erythrocytes (sRBC). Female B6C3F1 mice were treated orally for 3 days with vehicle or 3, 10, and 30 mg/kg SC-58236 or naproxen sodium. Maleic vinyl ether was used as positive control. Peripheral blood clearance of labeled sRBC was monitored for 60 minutes, then the animals were sacrificed, exsanguinated, and liver, kidney, spleen, lungs, thymus, skin, intestine and kidneys were removed and analyzed for radioactivity. The *in vitro* data showed no effect at any concentration with SC-58236 and a statistically significant inhibition of phagocytosis at all naproxen sodium concentrations tested. The *in vivo* study showed no effect of SC-58236 or naproxen sodium on phagocytic indices (organ weights, %uptake or total counts/mg tissue). Collectively, these data indicate that COX-2 is not involved in the phagocytic function of macrophages and that its inhibition at the site of inflammation does not affect the primary phagocytic activity of inflammatory cells.

1739 PERIPHERAL BENZODIAZEPINE RECEPTOR (PBR) EXPRESSION AS A MARKER OF LIPOPOLYSACCHARIDE (LPS)-INDUCED INFLAMMATION.

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Inflammatory diseases afflict millions of people each year. The ability to visualize the immune response with compounds targeted to immune cells will enhance our understanding and diagnosis of inflammatory disease. PBR is present in neutrophils and monocytes, as well as in microglia, the macrophages of the brain. Specific PBR ligands have been used to visualize brain tumors and inflammation using non-invasive imaging techniques such as positron emission tomography. To further develop the use of PBR as a non-invasive marker of inflammation, we used LPS as a model of inflammation in mice. Mice were injected with 0, 10, or 30mg/kg LPS. Two days later, mice were injected with the PBR specific radioligand 123I-(R)-PK11195. Static planar images revealed increased radioactivity in the chests of both 10 and 30mg/kg LPS animals (48±13% and 161±24%, respectively) above control levels. Immediately after the scan, animals were sacrificed and various organs harvested. In the heart, gamma counting revealed significant increases in radioactivity for both 10 and 30mg/kg LPS above controls (86±30% and 232±31%, respectively; $p<0.05$). In the lungs and spleen, only the 30mg/kg LPS mice were significantly increased above control levels (191±51% and 272±70%, respectively-$p<0.05$). To determine the specificity of the PBR response, 30mg/kg LPS mice were treated with excess non-radioactive (R)-PK11195 30 minutes prior to tracer injection. Static planar images revealed that activity in the chest of blocked animals was lower than in 30mg/kg animals (decreased 31±5%, $p=0.013$). Gamma counting revealed that specific tracer activity in the blocked animals was decreased in the lungs (39±7%; $p=0.023$) and spleen (54±6%; $p=0.012$). These data show that LPS induces specific increases in PBR ligand binding in the lungs and spleen. Further, this study indicates that PBR expression is a promising non-invasive marker for imaging inflammatory responses. [supported by grant # ES07062 to TRG]

1740 CRISTOBALITE AND αQUARTZ SILICA ACTIVATES NF-κB-DEPENDENT GENE TRANSCRIPTION IN MOUSE MACROPHAGES.

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Exposure *in vitro* of mouse macrophages to inorganic particles increases expression of several inflammatory cytokines whose promoter regions contain binding sites for specific transcription factors. Past studies by others have demonstrated the ability of crystalline silica particles to induce transactivation of the transcription factor, NF-κB (Rojanasakul et al; Mol Cell Biochem 200:119, 1999). However, few studies have evaluated particle characteristics responsible for this effect. To investigate if two forms of crystalline silica, αquartz and cristobalite, or amorphous silica (all ~1um) could activate NF-κB dependent gene transcription in mouse macrophages, RAW264.7 cells were transiently transfected (DEAE/chloroquine) with an NF-κB luciferase construct. The following day, cells were exposed to increasing concentrations of particle (6, 12 or 25 ug/cm^2) for 8 or 24 hrs. Both αquartz and cristobalite increased gene transcription by approximately 2 fold with a maximal activation at 25 ug/cm^2 for 8 hrs. Activation was specific for crystalline silica since exposure of cells to amorphous silica did not activate NF-κB dependent gene transcription. These data suggest that particle characteristics may influence transcription factor activity altering cytokine expression and eventual cell activation. (Funded by ES NIH09433).

1741 INHIBITION OF TNFα PROMOTER/ENHANCER ACTIVITY BY PHENOLIC ANTIOXIDANTS.

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Exposure to occupational chemicals often elicits inflammatory responses, resulting in tissue damage and disease. The cytokine Tumor necrosis factor alpha (TNFα) is an important mediator in the early stages of inflammation and plays an essential role in many inflammatory diseases. Certain phenolic antioxidants exhibit anti-inflammatory activity and thereby protect against chemical toxicity. The mechanism of anti-inflammation by the antioxidants is largely unclear. In a previously study, we demonstrated that phenolic antioxidants block signal-induced TNFα protein and mRNA production in macrophage cells. In this study, we further investigate the molecular mechanism of this inhibition by analyzing the effect of the antioxidants on the promoter/enhancer activity of TNFα. We used a stable reporter cell line that expresses luciferase under the control of TNFα promoter/enhancer for measuring TNFα gene transcription. The data reveal that bacterial endotoxin LPS induces luciferase production and tert-butylhydroquinone (tBHQ), a prototype of phenolic antioxidants, blocks this induction in the cell. The inhibition is both time- and dose-dependent. Inhibition of LPS-induced transcription of TNFα by diphenols (hydroquinone, HQ; catechol, and resorcinol) correlates with their capacity to undergo redox cycling. Furthermore, tBHQ and HQ blocked LPS-induced H_2O_2 generation within the cell, while resorcinol did not. Treatment with N-acetyl-cysteine, a general ROS inhibitor, blocks LPS-induced luciferase expression similarly. Taken together, these findings demonstrate phenolic antioxidants inhibit TNFα gene transcription and implicate redox cycling in the inhibition.

1742 EFFECTS OF INHALED CARBON BLACK PARTICLE OVERLOAD ON CYTOKINE, OXIDANT, AND MUTATIONAL RESPONSE IN THE LUNG: A SPECIES COMPARISON.

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Increased levels of adenomas and carcinomas in the rat lung have been associated with chronic inhalation to poorly soluble low toxicity particles at levels that cause persistent, marked inflammation. This response appears to be unique to the rat species and has not been observed in mice, or hamsters. Substantial evidence indicates this species specific lung tumor response is from a secondary, non-genotoxic, inflammatory mechanism. The recruitment of neutrophils during the inflammatory response and their subsequent release of oxidants from these cells have been demonstrated to play an important role in the pathogenesis of lung tumors. To investigate mechanisms underlying species specific lung inflammation and its persistence, female Fisher 344 rats and B6C3F1 mice were exposed to air, 1, 7 and 50mg/m3 of carbon black for 13 weeks. Serial sacrifices were performed immediately, 3 months and 11 months post exposure. Bronchoalveolar lavage (BAL) was performed and characterized for cell number, type, oxidant and antioxidant levels. *ex vivo* mutational analysis of inflammatory cells was evaluated by co-incubating with a lung epithelial cell line (RLE). Additionally, lung tissue was evaluated for gene expression and enzyme activity of various pro- and anti- inflammatory mediators. There was a dose and time related effect with all the parameters evaluated for both species. Rats showed a greater propensity for generating a pro-inflammatory response while mice demonstrated an increased anti-inflammatory response. These differences in the pro- and anti-inflammatory response may contribute to the apparent species differences in tumor response. Sponsored by the International Carbon Black Association.

1743 COMPARATIVE DOSE-RELATED EFFECTS IN THE LUNGS OF RATS AND MICE FOLLOWING CARBON BLACK INHALATION.

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High concentrations of inhaled carbon black (CB) induce overload-associated lung tumors in rats, but not mice or hamsters. The specificity of this response is not related to direct genotoxicity of CB particles, but may be related to species-dependent activation and progression of inflammatory and proliferative processes. In order to examine particle dose-related inflammation, responses to inhaled CB (1, 7, 50 mg/m^3, 13 wks) in the post-exposure phase have been compared in female F-344 rats and B6C3F1 mice. At the end of exposure, mice had retained more CB per gram of lung in terms of particle surface area than rats; however, clearance was slower in rats such that the CB burden in the high-dose group was higher than in mice 11 months post-exposure. Throughout the post-exposure phase, CB-induced increases in lung weights were comparable in rats and mice. For the low- and mid-dose groups, increases in total lavageable cells were also comparable in the two species; however, the total cell increases after high-dose CB in rats was greater and more prolonged. Lavage neutrophils increased in both species in response to mid- and high-dose CB; however, the response to the highest dose was ~3-10 times greater in rats than in mice. Increases in biochemical indices of inflammation were comparable in rats and mice at the two lower doses, but higher in rats at the highest dose. The results suggest that the lowest exposure dose of 1 mg/m^3 CB did not have any adverse effects in either species. In addition, rats appear to have greater and more prolonged inflammation in response to high-dose CB as well as retain CB longer. Funded by the International Carbon Black Association.

1744 INVESTIGATIONS ON THE INFLAMMATORY AND GENOTOXIC LUNG EFFECTS OF TWO TYPES OF ULTRAFINE TITANIUM DIOXIDE - UNTREATED AND SURFACE TREATED.

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TiO2 is considered to be toxicologically inert, at least under non-overload conditions. There is discussion about enhanced toxicity of ultrafine particles, independent of the particle chemistry. Therefore, we investigated inflammatory and genotoxic lung effects of two types of ultrafine TiO2 at low doses relevant for working environments. Female Wistar rats were exposed by instillation to a single dose of 0.15, 0.3, 0.6, and 1.2 mg of ultrafine TiO2 (P25, untreated) or ultrafine TiO2 (T805, surface treated) particles suspended in 0.2 ml of physiological saline containing 0.25 % lecithin. As controls, animals were instilled with the vehicle only or with a single dose of 0.6 mg quarz DQ12. 3, 21, and 90 days after exposure, 10 rats per group were sacrificed. In 5 animals bronchoalveolar lavage was performed and inflammatory parameters (cells, protein, TNF-alpha, fibronectin, surfactant phospholipids) were measured. The other animals' lungs were inflated with freezing medium, removed and stored in liquid nitrogen. For immunohistochemical staining, 8 µm frozen sections of the left lobe were cut and stored at -80°C. The sections were used for detection of 8-oxoguanine by a polyclonal antibody. Using a secondary fluorescence labelled antibody, 8-oxoguanine was quantified by image analysis. In the quarz exposed animals, a strong progression in lung inflammatory response was observed. 90 days after exposure, a slight but not significant increase in the amount of 8-oxoguanine in the DNA of lung cells was detected. In contrast, animals exposed to both types of TiO2 showed no signs of inflammation 90 days after exposure. The level of 8-oxoguanine as a marker of DNA damage was comparable to vehicle control, even in the highest dose. The results indicate the inert type of both titanium dioxides because neither any inflammatory nor genotoxic effects could be observed in the hygienic relevant range of doses.

1745 INFLAMMATORY TRANSCRIPT AND PROTEIN PRODUCTION BY CULTURED HUMAN KERATINOCYTES FOLLOWING SULFUR MUSTARD (SM) EXPOSURE.

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Human skin exposure to SM occurs initially without pain; however, over time, SM produces a painful burn with the loss of adherence of the basal keratinocyte layer to the dermis, which progresses to form large coalescing fluid-filled blisters. The primary changes occurring within the epidermal basal keratinocyte layer producing this loss of attachment are not known. However, in human skin, a strong delayed inflammation response occurs, which we believe enhances blister formation and tissue damage. We were interested in defining the specific inflammation transcript and protein changes produced by cultured human epidermal keratinocytes (HEK) following SM exposure. To focus these studies, we initially probed cDNA blots and produced subtraction libraries with cDNA generated from polyA transcripts isolated at various times (0.5, 1, 2, 4, 8 and 16 hr) after HEK exposure to SM. HEK exposure for these studies was performed either at 25 µM SM, an estimated subvesicating skin exposure, or at 200 µM, a vesicating exposure of SM. Using both techniques, we identified interleukin-8 (IL-8) as the predominant pro-inflammatory transcript up regulated in response to 200 µM SM. Transcripts also found using cDNA arrays were macrophage inflammatory protein-2α (MIP-α) and tumor necrosis factor-α (TNF-α). None of these transcripts was increased at the 25 µM SM exposure. Real-time PCR studies using an exposure range of 12.5-200 µM SM have identified transcript and protein increases in IL-8, TNF-α and MIP-α as early as 1-2 hrs for dose 100 and 200 µM, and the transcripts increase dramatically over time to 8 hr. Studies by ELISA showed parallel findings for protein production. These inflammatory transcript and protein data correlate well to doses estimated to blister skin and define the specific proteins that may be participating in the inflammatory events produced by HEK in exposed skin.

1746 FOUR WEEK INHALATION TOXICITY STUDY WITH NYLON RFP IN RATS.

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Groups of male rats were exposed, nose-only to aerosols of Nylon RFP (respirable-sized fiber-shaped particulates) for 4 weeks at concentrations of 0, 4, 15 and 57 f/cc (ratio of RFP: particles 1:10-20). The mean gravimetric counts were 0.6 (low), 2.7 (mid)and 19.6 (high level) mg/m3. The samples containing Nylon RFPs were prepared using flock rotary cutters. Subsequently, the lungs of sham and Nylon-exposed rats were assessed at 24 hrs, 1 week, and 1, 3, 6 and 12 months postexposure (pe) using bronchoalveolar lavage (BAL) and/or lung tissue analyses. Results showed that retained mean lung burdens at 24 hrs pe were 1.8E+07 RFP (high level) 3.4E+06 RFP (mid level) and 4.8E+05 RFP (low level). Mean lengths and diameters of the Nylon aerosol were 9.8 and 1.6 um, respectively. Six months pe, the retained lung burdens were 8.9E+05 RFP(high), 1.9E+05 RFP (mid), and 1.7E+05 RFP/lung (low level). There were no significant increases in lung weights or indications of pulmonary inflammation/cell proliferation in Nylon-exposed animals compared to controls - based on BAL cell differentials, BAL fluid analyses, BrdU cell proliferation (airway and parenchymal) and chemotaxis activity. Greater than 90% of the alveolar macrophages recovered by BAL (high level) contained Nylon RFP or

nonfibrous particulates within their cytoplasm. Interim histopathological analyses have thusfar (thru 6 months pe)revealed no adverse lower pulmonary or upper respiratory effects. These results indicate that 4-week inhalation exposures in rats to concentrations as high as 57 f/cc Nylon RFP produced no significant adverse pulmonary effects. (Study funded by the American Fiber Manufacturers Association).

1747 REMOVAL OF SOLUBLE METALS FROM RESIDUAL OIL FLY ASH BY CHELEX PROTECTED RATS FROM INCREASES IN MORTALITY AND LUNG INJURY AFTER INFECTION.

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Inhalation of residual oil fly ash (ROFA) impairs lung defense in susceptible populations. Bioavailability of soluble transition metals appears to play a key role in compromised lung defense caused by ROFA exposure. We evaluated the effect of soluble metals on lung defense in animals pre-exposed to ROFA followed by pulmonary challenge with a bacterial pathogen. ROFA was collected from Boston Edison, Co., Everett, MA, suspended in saline, incubated for 24 hr at 37°C, and separated into soluble (ROFA-Sol) and insoluble (ROFA-Insol) fractions. A portion of ROFA-Sol was treated with the metal-binding resin, Chelex, for 24 hr at 37°C. Sprague-Dawley rats were intratracheally dosed at day 0 with 1.0 mg/100 g body wt of ROFA-Insol, ROFA-Sol, saline, saline + Chelex, or ROFA-Sol + Chelex. At day 3, $5x10^5$ *Listeria monocytogenes* were intratracheally instilled into rats from each treatment group. At days 6, 8 and 10, left lungs were removed, homogenized, cultured, and colony forming units (CFUs) were counted to assess bacterial clearance. Treatment with ROFA-Sol before infection led to a marked increase in lung injury at all time points after inoculation, and a 50% increase in mortality in comparison to saline control rats. Treatment with ROFA-Insol, saline + Chelex, and ROFA-Sol + Chelex caused no significant increases in lung damage and mortality when compared to control. On day 6, a 34-fold increase in the number of bacterial CFUs was observed for the ROFA-Sol group when compared with saline control. By day 10, the ROFA-Sol group had 3693 times more lung CFUs than saline control, indicating its inability to effectively respond to the infection. None of the other groups had significant impairments in bacterial clearance. In summary, exposure to ROFA-Sol suppressed the lung response to infection. Upon removal of soluble metals from ROFA, there was no alteration in lung defense mechanisms. Soluble metals present in ROFA may play a key role in increased susceptibility to pulmonary infection in exposed populations.

1748 GENERATION OF METAL-INDUCED REACTIVE OXYGEN SPECIES BY RESIDUAL OIL FLY ASH.

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Inhalation of residual oil fly ash (ROFA) increases pulmonary morbidity in exposed workers. We examined the role of reactive oxygen species (ROS) in ROFA-induced lung injury. ROFA was collected from Boston Edison Co., Everett, MA. ROFA (ROFA-total) was suspended in saline, incubated for 24 hr at 37°C, centrifuged, and separated into soluble (ROFA-sol) and insoluble (ROFA-insol) fractions. The generation of ROS from the ROFA samples was measured by electron spin resonance (ESR). Sprague-Dawley rats were intratracheally instilled with saline, ROFA-total, ROFA-sol, or ROFA-insol (1 mg/100 g body weight). Lung tissue and bronchoalveolar lavage cells were harvested at 4, 24, and 72 hr after instillation. Chemiluminescence (CL) of recovered cells was measured as an index of ROS production, and tissue lipid peroxidation was assayed to assess oxidative injury. Using a spin trap (DMPO), hydroxyl radicals were detected in the ROFA-total sample. Significantly more hydroxyl radicals were measured in ROFA-sol as compared to ROFA-insol. Superoxide anion and hydrogen peroxide were implicated in the production of the hydroxyl radicals. After treatment with the metal chelator, desferrioxamine, the production of hydroxyl radical was greatly diminished. None of the ROFA samples had an effect on CL or lipid peroxidation at 4 hr. Treatment with ROFA-total and ROFA-insol caused significant increases ($p<0.05$) in both CL (at 24 hr) and lipid peroxidation (at 24 and 72 hr) when compared to control. ROFA-sol significantly reduced ($p<0.05$) CL production at 72 hr after treatment and had no effect on lipid peroxidation at any time point. In summary, ROFA, particularly its soluble fraction, generated a metal-dependent hydroxyl radical as measured by cell-free ESR. However, cellular oxidant production and tissue injury were observed mostly with the ROFA-total and ROFA-insol particulate forms. ROS generated by ROFA-sol as measured by ESR appear not to play a major role in the lung injury caused after ROFA exposure.

1749 REMODELING IN HUMAN LUNG IN RELATION TO PARTICLE AND METAL CONTENT.

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Little data exists about particle/metal burden in human lung and associated health effects. Remodeling of the terminal and respiratory bronchioles occurs at sites of particle deposition.We extend these findings providing data on particle and metal content in relation to indicators of effect classified by standard diagnostic criteria for 1)chronic bronchitis 2)asthma 3)mineral dust disease and 4)smoking-related disease.Lung autopsies(non-respiratory related deaths)were examined from 40 males from the Central Valley of California.Scanning electron microscopy was used to determine particle dose(No.particles/mg tissue) and inductively coupled plasma emission spectrometry for metal analysis.Lung samples with and without indicators of effect were compared.Significant($p<0.05$)fold increases in no. of particles was observed for indicators of mineral dust disease; (4.0X);lymph node fibrosis; (2.0X) smoking related disease (2.2X). No. of particles were (1.6X) lower in asthmatic lung samples. Regardless of disease state, >90% of the 24, 000 particles analyzed were <2.5 microns, 70% <1.0 and 42% <0.5. Ti, V and Mn were significantly $p<.05$ elevated in mineral dust disease and lymph node fibrosis. Consistent with tobacco contaminants, significant fold increases were shown for Ni(2.9X), Cr(3.6X), and Cd(4.6X)in smoking-related disease lung samples.Elevated metals were not observed in asthmatic lung tissue. Determining fate/dose of particulates in humans is essential for predicting health effects and elucidating the mechanisms of particle induced lung injury.Our data support the association between particle exposure and increased risk of lung disease.Microdisection, histology, evaluation of tissue changes and measurements of internal particle/metal burden provide a means toward establishing dose levels and anatomical sites in human lung required to produce adverse health outcomes.

1750 CARBONYL CONTENT OF DIESEL EXHAUST FROM TWO SOURCES AND POSSIBLE IMPLICATIONS FOR CELL RESPONSES.

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Diesel exhaust is known to cause health effects including lung inflammation and altered immunological parameters. The diesel exhausts used in our studies were collected into ice-cooled PBS from a diesel engine running at idle speed (DE2A) or at full load (DE5A). Previous work has shown that DE5A causes more cytotoxicity and greater increases in levels of inflammatory mediators in cultured human airway epithelial cells than DE2A. Some carbonyls are cytotoxic to epithelial cells and increase inflammatory mediator production. We therefore examined whether the carbonyl content of the exhausts were involved in the epithelial cell responses to diesel exhaust exposure. A method was developed for simultaneous determination of C_2 to C_{12} aldehydes by HPLC/MS. Diesel exhausts were derivatized with 2, 4-dinitrophenylhydrazine and derivatives isolated by hexane extraction and evaporation under nitrogen. Using quantitation of the peak at 365 nm in the UV/VIS spectra as an indication of the total concentration of carbonyl compounds in the reconstituted extracts, DE2A was found to contain a >10X higher level of total carbonyl compounds than DE5A, indicating an inverse correlation between toxicity and total carbonyl content. Reconstituted extracts were injected into an HPLC/MS with a C18 column using ESI negative ion detection with selective ion monitoring. HPLC/MS results were consistent with the UV/VIS result in that levels of polar aldehydes with retention times (R_T) similar to C_2 to C_4 averaged ~10X higher concentration in DE2A than in DE5A. However, DE5A contained higher levels of aldehydes of intermediate polarity with R_T similar to C_7 to C_{10} than did DE2A. These data suggest that diesel-derived carbonyls of intermediate polarity may be important in epithelial cell-mediated responses. These observations underscore the importance of sensitive and selective analytical methods such as HPLC/MS in linking components of complex mixtures to biological effects. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1751 DIFFERENTIAL PERSISTENCE OF ALVEOLITIS AND EPITHELIAL PROLIFERATION IN THE LUNGS OF RATS AND MICE EXPOSED TO CARBON BLACK.

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Rats, but not mice, chronically exposed to high levels of airborne carbon black particles (Cb) or other low toxicity particles develop lung tumors in association with chronic alveolar inflammation and epithelial proliferation. The purpose of the present study was to determine the postexposure persistence of alveolitis and type II cell

proliferation in the lungs of rats and mice similarly exposed to Cb. Female F344 rats and B6C3F1 mice were exposed by inhalation to three concentrations of Cb (1, 7, 50 mg/m3) for 6 h/day, 5 days/wk, for 13 wk. Rodents were sacrificed one day or 13 wk postexposure (PE). The left lung lobe of each animal was processed for light microscopy. Numeric cell densities of alveolar type II cells and neutrophils were determined using image analysis and standard morphometric techniques. Compared to air-exposed controls (0 mg/m3 Cb), rats exposed to Cb had a dose-dependent increase in numeric cell densities of neutrophils (alveolitis) and alveolar type II cells (epithelial hyperplasia). Cb-induced type II cell proliferation persisted throughout 13 wk PE only in the lungs of rats that were exposed to 50 mg/m3 Cb. In contrast, no or minimal Cb-induced alveolitis was evident in Cb-exposed mice at one day or 13 wk postexposure. A minimal increase in the number of alveolar type II cells was evident only in mice exposed to 50 mg/m3 Cb and sacrificed 13 wk PE. These results indicate that rats, but not mice, chronically exposed to high airborne concentrations of Cb develop severe and persistent alveolitis and epithelial proliferation for at least 13 wk PE. No persistent lung lesions were observed in mice or rats exposed to 1 mg/m3 Cb. (Research sponsored by the International Carbon Black Association)

1752 PARTICLE NUMBER AND SURFACE CHARGE PREDICT THE BIOLOGICAL ACTIVATION OF HUMAN BRONCHIAL EPITHELIAL CELLS EXPOSED TO PARTICULATE MATTER.

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Exposure to particulate matter (PM) produces a uniform degree of mortality in exposed populations, in spite of its diverse sources. This suggests a common mechanism of action to explain its initial toxicity. The present study relates certain physicochemical characteristics (i.e., size, number, surface charge, acidity) of PM particles to their biological activation (i.e., increases in intracellular calcium, $[Ca^{2+}]_i$, IL-6 release) in human, immortalized, tracheal bronchial epithelial cells (BEAS-2B). Urban ambient, volcanic, occupational and industrial PM were examined in suspension and after being passed through a 0.22 μm pore size, cellulose-acetate filter. The size, number and surface charge (i.e., zeta potential) of PM suspensions and their microfiltrates were measured using ZetaPALs technology. Linear regression analysis correlated the IL-6 release caused by field PM and their microfiltrates with their physicochemical characteristics. Of all of these, the surface charge (i.e., zeta potential) of particles in the suspended and microfiltrate fractions strongly correlated with the IL-6 released of their respective exposures. Similarly, the counts of microfiltrate particles also significantly correlated with IL-6 release associated with these fractions. To test whether the surface charge alone could stimulate cytokine release, polystyrene beads (SPM) of low and moderate zeta potentials were obtained. Exposing cells to 12-60 $\mu g/cm^2$ concentration of SPM produced IL-6 release similar to PM which was sensitive to CPZ exposure, an antagonist of the VR1 irritant receptor. RT-PCR indicated that the charged BEADS activated the acid sensitive, VR1 receptor of the BEAS-2B cells. Together, these data suggest that the surface charge of particles can stimulate a measurable biological response in human respiratory target cells. (This abstract has been reviewed by NHEERL but does not necessarily reflect EPA policy).

1753 A TYPICAL PATH MODEL OF TRACHEOBRONCHIAL CLEARANCE OF INHALED PARTICLES IN RATS.

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A typical path model of tracheobronchial (TB) particle clearance was developed using previously reported deposition model airway anatomy (Kimmel et al, 1998, Carpenter and Kimmel, 1999). Particle transport velocities for a typical airway in each generation were extrapolated from mean tracheal mucous transport velocities (TMV) using allometric equations describing the relationships between TMV and airway surface area, diameter, and length. Published interspecies allometric equations were modified to use theoretical (dimensional) exponents, indicating a greater degree of sameness, for intraspecies extrapolations. Multi-component clearance rate equations describing a typical clearance path from each generation of airways were developed. Consideration was given to conservation of mucous flow between airways. Overall TB region clearance was calculated by applying these clearance rate equations to the predicted fraction of aerosol particles deposited in corresponding airways generations. Predicted short-term clearance half-lives were 4.45, 6.42, and 20.97 hrs for aerosols in which theoretical deposition was 100, 80, and 50 % distributed evenly throughout the TB region. Although these initial predictions were in general agreement (within 15%) with published data they systematically overpredicted clearance. Adjustments of model clearance velocities for airway size dependent differences in mucous thickness and density and comprehensive comparison of model predictions with published data will be presented.

1754 THE EFFECT OF SIZE FRACTIONATED PARTICULATE MATTER ON HUMAN AIRWAY EPITHELIAL CELLS *IN VITRO*.

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Epidemiologic studies have demonstrated a compelling causal relationship between episodes of high concentrations of particulate matter (PM) and adverse health effects to the human respiratory and cardiovascular system, as evidenced by increased hospitalizations and mortality. Many factors have been implicated in the toxicity of PM, including particle size, number and constituents. Advances in technology now allow researchers to simultaneously collect, concentrate, and size fractionate coarse, fine, and ultrafine ambient air particulates. We have collected Chapel Hill ambient air PM in the following size ranges: coarse (CS) 2.5-10 micron, fine (FN) 0.1- 2.5 micron, and ultrafine (UF) <0.1 micron and examined their effects on human primary airway epithelial cells *in vitro* . Cells were exposed to 25, 100, and 250 ug/ml doses of each PM fraction for 2 and 24 hours. LDH and the inflammatory cytokine IL-8 were measured. Preliminary studies show that all three fractions elicit a dose-dependent response at 24 hours. When airway epithelial cells were exposed to different PM fractions on an equal mass basis, CS and UF fractions appeared to induce a larger cytokine response than FN particles. At 250 ug/ml, the highest dose examined, cells exposed to CS PM had approximately 4.3 fold greater IL-8 production than untreated cells; cells exposed to UF had 3.7 fold increased IL-8, while cells exposed to FN PM had 2.5 fold increased IL-8 compared to untreated cells. Similarily, human alveolar macrophages exposed 18 hours to 10 ug/ml CS and FN PM showed a far greater IL-6 cytokine production in response to coarse PM than fine PM (81.2 vs 7.8 fold increases compared to control). These studies suggest that coarse PM particles can induce increased inflammatory mediators in important lung cell types. [This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.]

1755 cDNA MICROARRAY ANALYSIS OF GENE EXPRESSION IN RAT ALVEOLAR MACROPHAGES IN RESPONSE TO ORGANIC EXTRACT OF DIESEL EXHAUST PARTICLES.

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Diesel exhaust particles (Department) induce pulmonary diseases including asthma and chronic bronchitis. The comprehensive evaluation has been required to know the effects of air pollutants including Department on the lung diseases. Alveolar macrophages (AM) encounter and phagocytose Department in the alveolar space and their biological responses are implicated in Department-induced pulmonary diseases. Therefore, we investigated the gene expression in rat AM after exposure to Department extract using a cDNA microarray. AM were prepared from male Sprague-Dawley rats (8-10 w) by bronchoalveolar lavage. The Department extract was prepared using dichloromethane. AM were pre-cultured for 20 h and exposed to 10 μg/ml Department extract for 6 h. Total RNA was extracted from AM using TRIZOL. cDNA probe synthesis and cDNA microanalysis were carried out using an Atlas Pure Total RNA Labeling System and Atlas Rat Toxicology Array II (CLONTECH) that includes 450 rat cDNAs. The results were further confirmed by northern blot analysis. Transcriptions of 6 genes on the cDNA microarray were significantly elevated by exposure to Department extract. These were heme oxygenase (HO) 1 and 2, thioredoxin peroxidase 2 (TDPX-2), glutathione S-transferase P subunit (GST-P), NAD(P)H dehydrogenase, and proliferating cell nuclear antigen (PCNA). The antioxidative enzymes such as HO, TDPX-2, GST-P, and NAD(P)H dehydrogenase may play a role in the pulmonary defense against oxidative stress caused by Department. PCNA may have contributed to the repair of the DNA damage and cell proliferation. Our results suggest that cDNA microarray analysis provides useful information to investigate the biological response to pulmonary toxicants.

1756 EFFECT OF EXTRACT OF PARTICULATE MATTER UNDER 2.5μM (PM2.5) AND DIESEL EXHAUST PARTICULATE (DEPARTMENT) ON RESPIRATORY AND CARDIAC FUNCTION IN SPONTANEOUSLY HYPERTENTION RATS.

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Epidemiological studies have shown the association between the morbidity and mortality of populations with preexisting cardiopulmonary disease and concentration of PM2.5. The present study was designed to clarify the effect of instillation of

PM2.5 on respiratory and cardiac function in rats with hereditary systemic hypertension. Male spontaneously hypertensive (SH) rats and Wistar/kyoto (WKY) rats, control strain, (11-12weeks old, 8/group) were used. Rats were operated to insert electrode subcutaneously. One week after, they were anesthetized with halothane, and were instilled intratrachealy with PM2.5 extract or Department extract (3mg/kg) in DMSO-PBS solution. Pulmonary function and electrocardiogram were measured at 3h before, 3h and 24h after instillation. R-R interval, power spectra of low frequency (LF) and high frequency (HF) were analyzed from electrocardiogram. LF/HF ratio and HF contents are indices of sympathetic and parasympathetic nervous activities, respectively. Lung resistance increased in SH and WKY rats administrated with PM2.5 or Department extract. In SH rats administrated with PM2.5, R-R interval extended, HF increased, but LF/HF didnÕt change. In WKY rats administrated with PM2.5, HF tended to increase, but R-R interval and LF/HF didnÕt change. Department, however, R-R interval, HF and LF/HF were not changed by Department in both SH and WKY rats. In conclusion, PM2.5 decreased heart rate and increased parasympathetic nervous activity in SH rats. Our results suggest that populations preexisting hypertension might be susceptible to PM2.5.

1757 LONG-TERM CARCINOGENICITY STUDY OF MAN MADE FIBERS BY INTRAPERITONEAL ROUTE IN RATS.

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The assessment of carcinogenicity of Man Made Fibers (MMF) in rats by the intraperitoneal (i.p.) route has been proposed as an alternative to inhalation studies, which may offer greater sensitivity. We have evaluated the carcinogenic potential of five vitreous MMF using this approach; MMF were administered as 2 to 20 weekly i.p. injections to groups of 51 female Wistar rats in a 123 week study. Crocidolite was used as a reference carcinogenic fiber. The tested fibers (designated M, O, P, V and B) were administered at 2 or 3 dose-levels up to 5 x 10^9 fibers, corresponding to an injected mass up to 400 - 700 mg. Crocidolite was given at dose-levels up to 1 x 10^9 fibers (up to 5 mg). The tested fibers did not produce notable adverse effects on the basis of mortality, clinical signs and body weight, except at the highest dose-level. All the fibers produced non-specific fibrous adhesions between abdominal organs, with dose-related severity; this finding was sometimes associated with chronic peritonitis or hepatocellular necrosis. Interestingly, the incidence of mesotheliomas permitted discrimination between the various fibers: - fibers M and O did not induce any mesothelioma at dose-levels up to 5 x 10^9 fibers; - fibers P and V induced mesothelioma in 16% and 28% of animals respectively at 5 x 10^9 fibers; - fiber B, for which previously published data was also available, induced mesothelioma in 18% of animals; - 90% of the crocidolite-treated animals displayed mesotheliomas at 1 x 10^9 fibers. No fiber-related tumor other than mesothelioma was observed. In conclusion, long-term bioassay in rats with i.p. injection was found to be a suitable and sensitive model which permitted discrimination of the carcinogenic potential of MMF with similar solubility or biopersistence.

1758 *IN VITRO* ASSAY OF THE BIOLOGICAL ACTIVITY OF AMBIENT $PM_{2.5}$ COLLECTED BY A HIGH FREQUENCY AEROSOL SAMPLER.

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Epidemiological evidence suggests that health effects associated with air particulate matter (PM) correlate closely with the $PM_{2.5}$ fraction, which is a highly complex, variable mixture of inorganic ions, elemental carbon, organic carbon compounds, trace elements and water. Local sources in urban environments contribute greatly to the composition of $PM_{2.5}$, thus studies of the specific components within this fraction that contribute to health effects are needed to develop effective control strategies. With this aim, an *in vitro* assay using A549 airway epithelial cells was developed to test the biological activity of $PM_{2.5}$ samples collected over time intervals as short as 1 hr with a recently developed high frequency semi-continuous aerosol sampler (SEAS) (Kidwell, C B and Ondov, J M, Development and Evaluation of a Prototype System for Collecting Sub-Hourly Ambient Aerosol for Chemical Analysis, Aerosol Science Technology 35: 596-601, 2001). Exposure of cells for 24 hr to a SEAS sample collected over a 24 hr period in College Park, MD produced no cytotoxicity (as measured by lactate dehydrogenase release) but particle concentrations from 12.5 to 100 µg/ml induced the release of IL8 in a dose dependent manner. Samples collected hourly at the same location differed over 10 fold in their ability to stimulate IL8 release, which ranged from 22 to 420 pg/ml of media. Cell responses to SEAS samples at 100 ug/ml were similar to those observed using NIST 1648 standard urban particulate matter at 200 µg/ml and 0.3 mM ZnCl2 exposures. Correlation of results of this assay with elemental analysis data for SEAS samples will aid in identifying the role played by trace elements in the toxicity of urban $PM_{2.5}$. *Supported by the Baltimore Supersite Program grant R82806301 and NIEHS Training Grant T32ES07263.*

1759 EFFECTS OF INSTILLED RESIDUAL OIL FLY ASH (ROFA) ON INDICES OF CARDIAC, PULMONARY, AND THERMOREGULATORY FUNCTION IN SPONTANEOUSLY HYPERTENSIVE (SH) RATS.

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Epidemiological studies have linked increased levels of ambient particulate matter (PM) with increased morbidity and mortality, noting higher correlations in persons with cardiopulmonary disease. To investigate this phenomenon, we examined the effects of ROFA (a model emission PM) in SH rats (a susceptible disease model). Rats were implanted with radiotelemeters capable of monitoring electrocardiogram (ECG), heart rate (HR), and core temperature (T_{co}). Animals were divided into four groups and exposed *via* intratracheal instillation (IT) to suspensions of ROFA (0.0, 0.25, 1.0, 2.5 mg) in acidified saline vehicle. Telemetered rats were monitored continuously for 96h post-IT and ventilatory function was examined (Buxco Electronics) for 6h/day on postexposure days 1 - 4. At 24h and 96h post-IT, subsets of rats underwent bronchoalveolar lavage (BAL) and the BAL fluid was examined for biochemical indices of pulmonary damage and inflammation. The IT exposures to vehicle and low dose ROFA caused no changes in T_{co}, while mid and high dose ROFA induced substantial decreases (1.2–2.6°C). ROFA-induced decreases in HR (30–120 bpm) did not return to control levels until 24h post-IT (low dose) or 72h post-IT (mid and high dose). ECG abnormalities (rhythm disturbances, premature ventricular contractions) were observed primarily in the high dose group. Adverse changes in BAL indices mirrored the dose-related changes in physiological parameters, yielding increases in protein, albumin, and LDH (24h post-IT>96h post-IT). These studies demonstrate substantial deficits in cardiopulmonary function in SH rats after IT exposure to ROFA PM, along with significant increases in BAL indices of pulmonary injury. Toxicity appears to be greater than that reported previously in healthy rats and comparable to that observed in other compromised rodent models. (Abstract does not represent USEPA policy.)

1760 *IN VITRO* EFFECTS OF PARTICULATE MATTER ON AIRWAY EPITHELIAL CELLS ISOLATED FROM CONCENTRATED AIR PARTICLES-EXPOSED SPONTANEOUS HYPERTENSIVE RATS.

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Spontaneously hypertensive (SH) rats, a strain with the genetic tendency to develop pulmonary and cardiac disease, are being used as a model to study the health effects of particulate matter (PM). In the present studies male SH rats were subjected to multiple exposures (12 exposures, over a 6 week period, 4 hr/day,) of either filtered air (control), or concentrated air particles (CAPS) from the Research Triangle Park (RTP), NC. Then, primary rat tracheal epithelial (RTE) cell cultures were established from both control and CAPS-exposed rats using an air-liquid interface system. After reaching confluency and maturity, cells were exposed to saline control, or to RTP-PM (PM 1.7-3.7, collected in bulk using a high volume sampler) at either 3.5 or 35 ug/ cm2 of culture area. Toxicity and cell injury were evaluated by lactate dehydrogenase (LDH) released and albumin translocation (index of increased solute permeability). Cell metabolism changes were evaluated by measuring intracellular glutathione (GSH) and ATP levels. RTE cell exposure to RTP-PM did not induce significant changes in LDH released by RTE cells. However, exposure of RTE cells to RTP-PM at 35 ug/ cm2 induced a mild increase in apical albumin levels that was greater in cells originating from CAPS-exposed rats than in cells from control air-exposed rats. RTP-PM exposures also induced decreases in GSH levels in cells originated from either air- or CAPS- exposed SH rats. RTP-PM exposures induced increases in ATP levels in RTE cells from either air or CAPS-exposed SH rats. Alterations of cellular metabolism by CAPS could contribute to the enhanced responses of cardiopulmonary compromised animals to the effects of ambient air pollutants, however more studies are needed to elucidate the extent of these differences. (The contents of this abstract do not necessarily reflect the views and policies of the USEPA).

1761 HEALTH EFFECTS OF CONCENTRATED CALIFORNIA PARTICULATE MATTER IN RATS.

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Epidemiological studies have shown that airborne particulate matter (PM) with an aerodynamic diameter less than 10 microns is associated with increased pulmonary disease. However, particles less than 2.5 microns are thought to elicit the greatest

toxicity. PM is a complex mixture of organic and inorganic compounds, but the properties responsible for its effects on the respiratory system remain unknown. During the fall and winter of 2000-01 adult Sprague-Dawley rats were exposed in Fresno, CA to filtered air or combined ultrafine and fine fractions of PM enhanced approximately 20-fold over ambient levels in 6 separate experiments. Exposures were for 4 hours per day for 3 consecutive days. PM was concentrated while preserving chemical composition, size distribution and surface morphology. PM mass and particle numbers were measured continuously during exposure. Chemical analysis of the concentrated PM was conducted for each study. The mean mass concentration of particles ranged from 200 to 900 micrograms/cubic meter. PM was enriched primarily with nitrate, organic and elemental carbon, and metals. Bronchoalveolar lavage (BAL) was performed on each group of rats following exposure. Viability, total cells, and numbers of macrophages, neutrophils and lymphocytes in the BAL was determined. Viability of BAL cells from rats exposed to concentrated PM was significantly decreased during 4 of 6 weeks, compared to rats exposed to filtered air ($P<0.05$). Total BAL cells were increased during 1 week of the 6-week study, while macrophages increased during 1 week and neutrophils during 2 weeks. The decreased cell viability in PM-exposed rats is indicative of cell membrane damage, possibly through oxidative stress catalyzed by transition metals. The increased neutrophils in PM-exposed rats indicates a mild inflammatory response. These observations suggest that exposure to enhanced concentrations of ambient particles in Fresno, CA is associated with significant effects in the lungs of healthy adult rats.

1762 EVALUATION OF TOXICITY BY OIL FLY-ASHES IN RAT ALVEOLAR EPITHELIAL CELLS: ROLE OF FENTON DRIVEN OH-RADICAL GENERATION AND PARTICLE FRACTION.

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Leachable transition metals are considered to be important mediators in the biological effects of ambient particulate matter (PM) and model particles such as oil-fly ashes (OFA). We determined metal driven Fenton activity in 10 OFA in the presence of hydrogen peroxide using electron spin resonance (EPR) and a suitable spin-trap (DMPO) and their ability to induce 8-hydroxydeguanosine (8-OH-dG) in nude DNA using immunodotblot. In addition, the toxicity of the OFA suspensions and supernatants was determined in a rat alveolar type II cell line using leaching of LDH (membrane damage) or conversion of MTT (mitochondrial function). Suspensions of OFA were consistently more toxic than their filtrates obtained by centrifugation and 0.1 µm filtration. Correlations between both cytotoxicity assays were seen using OFA suspensions ($r=0.862$) but not for the filtrates. The DMPO-OH generation of filtrates in water did correlate well with the cytoxicity assessed by MTT conversion of the filtrate ($r=0.820$) and the formation of 8-OHdG ($r=0.57$). Together these findings suggest that the different fractions of OFA exert cytotoxicity through different mechanisms. This is supported by different effects of filtrate and suspensions on 8-OHdG formation and DNA unwinding. The watersoluble Fenton-active fraction seems to affect mitochondrial competence (MTT), while the membranolytic effects seem to be determined by the total suspensions.(This abstract does not reflect USEPA policy).

1763 COMPARISON OF ASBESTOS FIBER CONTENT IN LUNGS OF CANCER PATIENTS WITH NORMAL KOREAN SUBJECTS WITH NO KNOWN OCCUPATIONAL ASBESTOS EXPOSURE HISTORY.

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To evaluate the effect of environmental asbestos exposure on inducing lung cancer, pulmonary fiber contents of both asbestos and non-asbestos types were evaluated in 36 normal Korean subjects and 37 lung cancer patients with no known occupational history of asbestos exposure. Pulmonary fiber contents were analyzed by transmission electron microscopy with energy dispersive X-ray analysis after using the low temperature ashing procedure. Chrysotile fiber was the major fiber type found in the lungs of Korean subjects. Asbestos fiber concentrations found in the lungs of normal 25 males and 11 females were 0.23 x 106 fibers/g of dry lungs and 0.15 x 106 fibers/g of dry lung tissue, respectively. Asbestos concentrations found in the lungs of cancer patients were 0.16 x 106 fibers/g of dry lungs for 31 males and 0.45 x 106 fibers/g of dry lungs for 6 females. There was no statistical difference in pulmonary asbestos content between normal and lung cancer patients.

1764 YOU CAN'T DILUTE FRESH COMBUSTION EMISSIONS WITHOUT ALTERING THEIR COMPOSITION.

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Recent research has shown that the dilution level can affect the both the particle size distribution and composition of diesel exhaust. This may be important in inhalation toxicology because lung deposition patterns may differ with changes in particle size distribution, and changes in chemistry can lead to non-linear dilutions of all constituents of combustion material such as diesel exhaust. Data reported here were generated during a diesel exhaust inhalation toxicology study conducted at Lovelace Respiratory Research Institute under the auspices of the National Environmental Respiratory Center (NERC). As part of this research, the size distribution and chemical composition of particulate material was investigated at four different dilutions of exhaust. Dilutions with scrubbed ambient air were set to achieve diesel particulate concentrations of 30, 100, 300, and 1000 microgram per cubic meter. Particle size distribution, including number count and mass distribution, were characterized at all exposure levels along with the chemical composition of the particulate material. Particle mass distribution centered at approximately 150nm for all exposure levels, but number distribution was different at all levels, with the highest dilution levels (lowest mass) producing higher amounts of particles in the "nanoparticle" range (<100nm). Particle composition was found to change most at the more concentrated levels, with chemical interactions between nitrogen oxides and ammonia producing higher relative levels of ammonium nitrate/ammonium sulfate particles at low dilution levels. Research conducted by the National Environmental Respiratory Center (NERC) with support from multiple government and industry sponsors, including the USEPA. This abstract is not intended to represent the views or policies of any NERC sponsor

1765 EFFECT OF ULTRAFINE CARBON BLACK EXPOSURE ON RESPIRATORY SYNCYTIAL VIRUS INFECTION IN MICE.

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Epidemiological studies have indicated that exposure to elevated levels of ultrafine (UF) particulate matter exacerbates a variety of pulmonary diseases, including asthma, bronchitis, and viral infections. Respiratory syncytial virus (RSV) is the most important etiological agent in respiratory diseases of infancy, is the major cause of bronchiolitis and pneumonia in infants under 1 year of age, and may lead to the development of asthma in childhood. RSV also contributes to the development of pneumonia in the elderly and the immunocompromised. To ascertain whether particle exposure modulates the immune response to RSV, we developed a BALB/c mouse model of RSV infection that mimics the pathophysiological and immunological features of RSV disease in humans. For the study, 8 week-old female BALB/c mice were instilled *via* the trachea with either 40 µg UF carbon black (CB) particles or saline vehicle. The following day, mice were instilled with either 10^6 pfu RSV or uninfected media. End points were examined 1, 2, 4, 7, and 10 days after RSV infection. Airways hyperresponsiveness and total IgE in the serum were increased in RSV-infected animals compared to controls and were unaltered by CB exposure. RSV antigen titers in the bronchoalveolar lavage fluid (BALF) and lung homogenates were reduced in RSV+CB-treated mice relative to RSV alone. On days 1 and 2 of infection, TNF-αprotein was reduced in the BALF of RSV+CB mice; on day 4, there was a significant reduction in BALF lymphocyte numbers, which correlated with a reduction in IFN-γprotein in the BALF of RSV+CB mice compared with RSV alone. Ribonuclease protection assays of lung tissue showed reductions in RANTES, eotaxin, MCP-1, MIP-1α, MIP-1β, and IFN-γand increases in IL-6, IL-10, and IL-15 message in RSV+CB mice compared to RSV alone. These data indicate that preexposure to CB particles may stimulate alveolar macrophages and the pulmonary immune response to RSV infection, leading to accelerated viral clearance and less severe disease.

1766 ROLE OF ASSOCIATED TRANSITION METALS IN THE IMMUNOTOXICITY OF INHALED PARTICULATE AIR POLLUTION.

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Epidemiological studies demonstrate that pneumonia contributes to increased mortality among individuals following exposure to particulate matter (PM). This suggests that PM acts as an immunosuppressant that undermines normal lung immu-

nity. A 5h inhalation exposure of bacterially-infected rats to concentrated ambient $PM_{2.5}$ from NYC air, altered immune function and exacerbated infection. *S. pneumoniae*-infected rats exposed to PM 48h following bacterial instillation had increased: bacterial burdens; circulating WBC; bacteria-associated lung lesions; and, bacteremia compared to controls. PM exposure also decreased lavageable PMN, cytokines, and BALT. While these results provide a basis for the epidemiological findings, the identity of constituents responsible for the worsening pneumonia remains unclear. To address this, studies were performed to correlate physiochemical attributes of ambient $PM_{2.5}$ with *in vivo* immunotoxicity. Previously-infected or uninfected rats were exposed (nose-only) to soluble forms of Fe, Cu, Mn, or Ni. At designated timepoints, uninfected rats were sacrificed, their lungs either lavaged for levels of cytokines, markers of lung damage, and oxyradicals, or fixed for histology. Blood cell profiles and B- and T-cell proliferation were also examined. In studies employing infected hosts, lungs were assessed for bacterial clearance and analyzed for extent/severity of infection. Results demonstrated that while exposure to Mn and Ni at ~90μg/m^3 had no effect on immunity, inhalation of Fe altered immune function in uninfected hosts and reduced pulmonary bacterial clearance in infected rats. Fe-induced changes in bacterial burdens correlated with elevated blood PMN and increased amounts of pneumococcal-associated lung lesions. Studies demonstrate that exposure to ambient PM worsens the outcome of an ongoing pulmonary infection, and that associated Fe may play a role in this immunotoxicity. EPA R827351 and HEI 95-14.

1767 CORRELATED PULMONARY AND SYSTEMIC EFFECTS IN SPONTANEOUSLY HYPERTENSIVE RATS FOLLOWING ACUTE EXPOSURES TO CONCENTRATED AMBIENT PARTICLES (CAPS).

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The increase in plasma fibrinogen, a risk factor for cardiovascular disease (CVD), has recently been associated with exposures to particulate air pollution (PM) in humans. We have shown a similar pattern of fibrinogen increases in rats exposed to emission PM. The purpose of this study was to ascertain the consistency of the plasma fibrinogen increase following acute CAPS exposures, and its relationship to lung injury in a rat model of CVD. Male Spontaneously Hypertensive (SH) rats (13 wks) were exposed whole body to filtered air (n=4-7) or CAPS (n=5-9) from local air, 4 h/d x 1 d (6 replicates) or 2 d (2 replicates). The estimated daily CAPS concentrations ranged from 700-1800 μg/m3. To assess injury, bronchoalveolar lavage fluid (BALF) protein, n-acetylglucosaminidase, inflammatory cells and antioxidants (BALF and lung), as well as plasma fibrinogen were analyzed either immediately or 20 h following final exposure. A small increase in plasma fibrinogen was apparent after each 2 d exposure, and in five out of six 1 d exposures. BALF neutrophils and lung cell injury parameteres did not indicate a consistent exposure-related pattern of response. However, the lung glutathione and ascorbate content decreased after both 2 day exposures, with a smaller decrease after 1 d exposure. The degree of change in lung injury and systemic parameters following the 2 d exposures appeared to be concentration-dependent, however, the fibrinogen increases noted in the 1 d exposure groups did not relate to CAPS concentration. The chemical composition of filters and meteorological data in relation to animal responses are being evaluated. This study demonstrates that acute CAPS exposure may produce antioxidant decreases in the lung and systemic increases in plasma fibrinogen in a rat model of human CVD. (This abstract does not reflect USEPA policy).

1768 EXPRESSION AND NUCLEAR TRANSLOCATION OF NF-E2 RELATED FACTOR (NRF2) IN HEPATIC STELLATE CELLS.

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Increased intracellular levels of reactive oxygen intermediates, or oxidative stress, can result from exposure to alcohol, iron or chlorinated hydrocarbons and is causally associated with hepatic fibrosis. During oxidative stress electrophilic intermediates, including 4-hydroxynonenal (4-HNE), are generated and *in vitro* purportedly stimulate hepatic stellate cells (HSC) to transdifferentiate from a quiescent to an activated phenotype. It is this activated phenotype that is responsible for the production of the excessive extracellular matrix characteristic of fibrosis. However, it remains unclear whether HSC activation is modulated directly by peroxidative intermediates *in vivo*, or through other cell signaling pathways. The objective of the present study is to investigate the role of oxidative stress in HSC activation. Recent studies have demonstrated that the bZIP transcription factor Nrf2 is crucial for antioxidant response element (ARE)-mediated gene transcription of phase II metabolizing enzymes. Electrophilic agents trigger the release Nrf2 from its cytoplasmic anchor protein (Keap1) allowing Nrf2 to translocate into the nucleus and activate ARE-dependent genes. In this investigation we have isolated and identified HSC for the purpose of studying oxidative stress induced HSC activation in rat liver following 4 weeks carbon tetrachloride (CCL4) treatment. In treated animals it is shown that HSC become activated and proliferate throughout the liver forming intralobular bands. These bands of activated stellate cells are further demonstrated to colocalize with bridging bands of fiberous tissue. Utilizing both freshly isolated and primary culture HSC we demonstrated that HSC express the Nrf2 transcription factor. Functional activity of Nrf2 was confirmed in cultured HSC suggesting its usefulness as a sensor system for oxidative stress *in vivo*. The studies presented here provide a foundation to clarify the role of oxidative stress in hepatic stellate cell activation *in vivo*. (Supported by NIH/NIAAH-09300 and F31 NIH/AA05578-01.)

1769 NITRIC OXIDE GENERATION BY CULTURED RAT HEPATOCYTES DURING SIMULATED ISCHEMIA.

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BACKGROUND: Nitric oxide (NO) is an important regulator of biological functions that has also been linked to cellular injury. NO can be generated during ischemia/reperfusion, but mechanisms of NO formation in ischemic hepatocytes are not known. The **AIM** of this study was to investigate the effect of ischemia on the generation of NO in hepatocytes. **METHODS:** Rat hepatocytes were cultured overnight and loaded with 10 μM DAF-FM diacetate, an NO specific fluorescent probe, for 30 minutes at 37°C in Krebs-Ringer-HEPES (KRH) buffer at pH 7.4. After washing three times with cold KRH, hepatocytes were further incubated for 15 minutes at 37°C to complete deesterification. The cells in warm KRH were then incubated in air (normoxic) or argon (ischemic) at either pH 6.2 or at pH 7.4 for up to 4 h. Changes in green DAF-FM fluorescence were monitored in a fluorescence plate reader. In other experiments, 5 mM arginine, 1 mM of the NO synthase inhibitor N-nitro-arginine methyl ester hydrochloride (L-NAME), or 100 μM of the NO donor S-nitroso-N-acetylpenicillamine (SNAP) was included in the incubation medium. Confocal images of DAF-FM fluorescence were collected with a Zeiss LSM 410 microscope. **RESULTS:** NO formation, as revealed by DAF-FM fluorescence, increased during anoxia at pH 6.2 but not at pH 7.4. NO production during 4 h anoxia at pH 6.2 was about 10-fold greater than during 4 h of normoxia at pH 6.2. Incubation of hepatocytes with arginine or L-NAME did not increase or decrease NO formation during ischemia, although SNAP rapidly enhanced DAF-FM fluorescence both in normoxic and ischemic cells at either pH. Confocal imaging confirmed a dramatic increase of intracellular DAF-FM fluorescence during ischemia but not during normoxia. **CONCLUSION:** Anoxia to hepatocytes under acidotic conditions enhanced NO formation. The oxygen-independent mechanism by which NO is formed during ischemia appears not to involve nitric oxide synthase. The contribution of NO formation to ischemic hepatocellular injury remains to be determined.

1770 TRANSLATIONAL REGULATION OF RAT HEPATIC MRP2.

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Mrp2 mediates the biliary excretion of glutathione, glucuronide and sulfate conjugates. Hepatic expression of Mrp2 protein is decreased in pregnant (P) rats but is increased in rats treated with pregnenolone-16α-carbonitrile (PCN), eventhough expression of Mrp2 mRNA is unchanged. We examined hepatic expression of Mrp2 protein (Western analysis), mRNA (Northern analysis) and the polysomal distribution of Mrp2 mRNA in female control (C) and P (19 - 20 d) rats, and in rats treated with corn oil (CO) or PCN (75 mg/kg/d, 2d, ip). Polyribosomes (polysomes) were separated from single ribosomes (monosomes, M) by differential sedimentation through a continuous sucrose gradient (20-75%), followed by Northern analysis of Mpr2 mRNA purified from 24 individual fractions (0.5 ml) of the sucrose gradient. Results are expressed as "% of total Mrp2 mRNA" in the bottom (I; 1-7), middle (II; 8-14) and top (III; 15-21) of the gradient and the M (22-24) fractions and show Mean ± SE where n = 3-5; *, $p < 0.05$ in C vs P or CO vs PCN, t- test. RESULTS. Mrp2 protein was decreased by 50% (*) in P vs C and increased two-fold (*) in PCN vs CO; mRNA was unchanged. In polysomal distribution analysis (Table), C Mrp2 mRNA was uniformly distributed throughout the gradient. In P, Mrp2 mRNA shifted from I, containing the heavier polysomes, to II and III, relative to C. In PCN, Mrp2 mRNA in I was increased two-fold relative to Co., reflecting a shift from II and III. CONCLUSIONS. The decreased enrichment of Mrp2 mRNA in the denser polysomal fractions in P is consistent with a decrease in protein synthesis and decreased Mrp2 protein expression while increased association of Mrp2 mRNA with the fraction most actively engaged in protein synthesis is consistent with increased Mrp2 protein expression in PCN. These

data indicate that in P and PCN rats, hepatic Mrp2 expression is translationally regulated.

Fraction	C	P	CO	PCN
I	25.5±3.9	8.0 ±1.5*	23.8 ±4.8	47.3±6.2*
II	34.0±3.0	46.1±2.6*	25.0±3.2	17.4±4.4
III	23.4±3.3	40.5±2.3*	45.2±5.0	29.1±4.7
M	16.8±3.9	5.4±4.1	6.0±3.2	6.2±2.0

1771 STRUCTURAL DETERMINANTS OF PERFLUOROOCTYL-DERIVATIVE-MEDIATED INDUCTION OF THE MITOCHONDRIAL PERMEABILITY TRANSITION.

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Perfluorooctanoate (PFOA), perfluorooctanesulfonate (PFOS), and substituted perfluorooctanesulfonamides are commercial compounds widely used as water and stain repellents, fire retardants, and anti-corrosion agents among many other applications. We recently showed that these compounds disrupt mitochondrial bioenergetics in one of three ways: by stimulating proton conductance to uncouple mitochondrial respiration, as inducers of the mitochondrial permeability transition (MPT), or by causing the nonspecific increase in ion permeability across the mitochondrial membranes. The purpose of this investigation was to screen several members of this class of compounds, PFOA, PFOS, perfluorooctanesulfonamide (FOSA), perfluorooctanesulfonamidoacetare (FOSAA), N-ethylperfluorooctanesulfonamide (N-Et-FOSA), 2-(N-ethylperfluorooctanesulfonamido)ethyl alcohol (N-Et-FOSE), and N-ethylperfluorooctanesulfonamidoacetate (N-Et-FOSAA) to reveal a common structural characteristic responsible for induction of the mitochondrial permeability transition. Each compound was added at concentrations previously shown to alter mitochondrial respiration to a reaction medium containing glutamate/malate energized rat liver mitochondria. Mitochondrial swelling was monitored spectrophotometrically. PFOS, FOSA, N-Et-FOSA and N-Et-FOSE did not induce swelling at any concentration. In contrast, the carboxylated compounds PFOA, FOSAA, and N-ET-FOSAA induced calcium-dependent, cyclosporin A inhibited mitochondrial swelling. The fully substituted amide N-Et-FOSAA is the most potent inducer of the MPT, followed by FOSAA, then PFOA. The results indicate that the primary determinant of induction of the MPT is the carboxylic group, and that it is the induction of the MPT that is responsible for the observed interference with mitochondrial respiration by these carboxylated fluorochemicals. (This work as supported by a grant from the 3M Company).

1772 THE IMPACT OF PERFLUOROOCTYL DERIVATIVES ON PEROXISOME PROLIFERATION AND MITOCHONDRIAL BIOGENESIS.

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Perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA) and related sulfonamido derivatives have drawn recent attention due to their broad industrial application, environmental persistence, and detection in biological samples. Despite their chemical stability, these compounds induce peroxisome proliferation characterized in part by the upregulation of enzymes of fatty acid β-oxidation. Several of these fluorochemical derivatives interfere with mitochondrial bioenergetics *in vitro* and PFOA is capable of stimulating both peroxisomal and mitochondrial proliferation *in vivo*. The purpose of this investigation was to determine whether stimulation of mitochondrial biogenesis is a general characteristic associated with proliferation of peroxisomal bodies in liver. PFOS and N-ethyl perfluorooctanesulfamido ethanol (N-EtFOSE) were investigated as potential peroxisome proliferators using PFOA as a positive control. PFOA, PFOS, or N-EtFOSE was administered *via* a single IP injection at 100mg/kg to adult male rats and measurements were made 3 days later. Parameters chosen to assess peroxisome proliferation were; stimulation of lauroyl CoA oxidase activity, reduction of serum cholesterol, and hepatomegaly. Mitochondrial biogenesis was assessed on the basis of cytochrome oxidase activity, cytochrome content, and mitochondrial DNA copy number (mtDNA). In this model, PFOS and PFOA shared similar peroxisome proliferative activity, whereas N-EtFOSE did not induce peroxisome proliferation. Mitochondrial parameters were altered only in the PFOA group where mtDNA was increased and cytochrome c oxidase activity was decreased in liver tissue. None of the compounds tested affected cytochrome content. These results demonstrate that acute treatment with PFOS or PFOA causes hepatic peroxisome proliferation in rats. However, induction of mitochondrial biogenesis appears to be a characteristic of PFOA and not an effect associated with the peroxisome proliferative response in general. (Supported by the 3M Company.)

1773 INDUCTION OF HEMEOXYGENASE-1 (HO-1) PROTECTS AGAINST TNF-INDUCED APOPTOSIS AND NEUTROPHIL-MEDIATED NECROSIS IN THE LIVER.

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HO-1 is a stress-response enzyme, which can protect against oxidative tissue injury. We previously demonstrated the critical role of reactive oxygen in the model of galactosamine/endotoxin-induced apoptosis and neutrophil-mediated liver cell necrosis (Hepatology 29: 443-450, 1999). To investigate a potential protective role of HO-1, C3Heb/FeJ mice were pretreated with 5 or 15 mg/kg Cobalt-protoporphorin IX (CoPP). This treatment dose-dependently induced HO-1 in nonparenchymal cells and in hepatocytes as assessed by immunohistochemistry and Western blotting of isolated cell fractions. Injection of 700 mg/kg galactosamine and 0.1 mg/kg endotoxin (G/E) caused hepatocellular apoptosis as indicated by a 10-fold increase of caspase-3 activity and procaspase-3 processing at 6 h. Large numbers of neutrophils were present in sinusoids and postsinusoidal venules at that time. Following transmigration, these neutrophils caused additional cell injury as indicated by high levels of ALT (3720 ± 920 U/L) and necrosis (40-50%) at 7 h. Both doses of CoPP signifcantly attenuated the increase in plasma ALT levels (5 mg/kg: 244 ± 120 U/L; 15 mg/kg: 51 ± 14 U/L) and necrosis (10%) but had no effect on hepatic neutrophil sequestration in either vascular bed. Caspase-3 activity was reduced by 40% and 90% with the low and high dose of CoPP, respectively. Additional treatment with the HO-1 inhibitor Sn-PP restored the sensitivity to G/E-mediated injury. Cell injury after injection of 700 mg/kg galactosamine/7 μg/kg murine TNF-α was also attenuated with HO-1 induction. CONCLUSION: Induction of HO-1 in nonparenchymal cells and in hepatocytes with CoPP dose-dependently protected against TNF-mediated apoptotic cell death and subsequent neutrophil-induced injury to hepatocytes. The mechanism may involve the increased formation of endogenous antioxidants by HO-1.

1774 ACETAMINOPHEN TOXICITY AND APOPTOTIC SIGNALING PATHWAYS IN THE LIVER.

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Recently, it has been shown that a moderately toxic dose of acetaminophen (AAP) interrupts the signaling pathway of Fas receptor-mediated apoptosis (Toxicol Appl Pharmacol 156: 179-186, 1999). The aim of our study was to investigate the mechanism of this effect. Male C3Heb/FeJ mice received a single dose of AAP (300 mg/kg i.p) and/or anti-Fas antibody Jo-2 (0.6 mg/kg, iv). Some animals were treated with allopurinol (100 mg/kg, po) 18 and 1 h before AAP injection. RESULTS: After 90 min of Jo-2 treatment, there was processing of BID and procaspase-3 and a significant increase in liver caspase-3 activity, which is consistent with apoptotic cell death. Treatment with AAP 2.5 h before Jo-2 prevented the increase in hepatic caspase-3 activities and the processing of both BID and procaspase-3. When administered alone, AAP did not cause processing of BID or procaspase-3 but caused significant liver injury. AAP metabolism resulted in a substantial depletion of tissue and mitochondrial glutathione (GSH). However, the mitochondrial GSSG levels increased from 3-5% of total glutathione in controls to 15-25% 1 - 4 h after AAP indicating a significant oxidant stress. Pretreatment with allopurinol prevented the mitochondrial oxidant stress and liver injury due to AAP toxicity but had no effect on Jo-mediated apoptosis. Consequently, allopurinol restored hepatocyte sensitivity to Fas receptor signaling in AAP-treated animals. Evaluation of DNA fragmentation with the TUNEL assay showed staining of midzonal (Jo-2) or centrilobular hepatocytes (AAP). Treatment with AAP eliminated staining of midzonal hepatocytes after Jo-2 injection. Allopurinol restored TUNEL staining of midzonal and prevented staining of centrilobular hepatocytes after combined Jo-2 and AAP administration. CONCLUSION: Metabolic activation of AAP causes mitochondrial dysfunction, which is responsible for disruption of Fas receptor-mediated intracellular signaling. These data suggest that maintaining mitochondrial integrity is critical for the execution of receptor-mediated apoptotic cell death pathways in hepatocytes.

1775 MECHANISMS OF PROGRESSION AND REGRESSION OF ACUTE LIVER INJURY.

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Toxicant-initiated liver injury progresses to liver failure unless newly divided cells obtund progressing injury. To test the hypothesis that progression of injury occurs due to destructive action of cytoplasmic and lysosomal degradative enzymes (death proteins) released from dying cells, the role of calpain, a Ca^{2+}-activated cytoplasmic

protease was evaluated. A specific calpain inhibitor, N-CBZ-VAL-PHE- methyl ester (CBZ, 60 mg/kg, in DMSO), administered ip 1 h after a lethal dose of CCl_4 (3 ml/kg, in corn oil, ip) protected 75% of male S-D rats. Liver injury measured by ALT was 6-fold lower in the rats receiving CBZ. Calpain leakage from dying cells was confirmed by immunohistochemistry in liver sections at 0 to 150 h after CCl_4 injection (2 ml/kg, in corn oil, ip). Calpain appeared in the perinecrotic intercellular spaces at 3 h after CCl_4 and increased up to 48 h spearheading progression of injury. In the CCl_4 + CBZ treated group, calpain leakage and liver injury were strikingly lower. Plasma calpain activity after CCl_4 treatment mirrored the intercellular leakage and progression of injury, while it was lower in the inhibitor treated group mirroring lower injury. Breakdown of α- fodrin, a cytoskeletal protein, and specific calpain substrate, analyzed in liver samples by Western blot, increased at 12 and 48 h after CCl_4 treatment. Normal rat hepatocytes incubated with calpain and 1.3 mM free Ca^{2+} confirmed that calpain can induce cell death. In contrast, dividing cells remain unaffected by destructive action of death proteins due to higher expression of endogenous inhibitors. Immunohistochemically, expression of calpastatin, the endogenous inhibitor of calpain, was found to be increasing in dividing cells. These data indicate that calpain released from dying cells destroys cellular cytoskeleton, inducing cell death and progression of liver injury, while higher expression of calpastatin in newly divided cells resists its action and thereby regresses injury.

1776 INDUCTION OF RAT LIVER MULTIDRUG RESISTANCE PROTEIN 3 (MRP3) EXPRESSION FOLLOWING TREATMENT WITH CARBON TETRACHLORIDE (CCl4) AND ACETAMINOPHEN (APAP).

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Chemically-induced compensatory hepatocellular proliferation results in enhanced protection against subsequent toxicant challenge. Induction of liver plasma membrane ATP-dependent transporters may be one of several mechanisms by which proliferating hepatocytes acquire enhanced resistance to cytotoxicity. The multidrug resistance associated proteins are one family of these transport proteins for organic anions. Within this family, Mrp3 is known to be inducible. Therefore, the relative abundance of Mrp3 mRNA transcripts was measured using branched DNA signal amplification. Male Wistar rats received either 900 mg APAP/kg i.p. or 26 µl CCl4/kg i.p. and control rats received vehicle only. Liver samples were collected at 6, 24, 48, and 72 hours after treatment and processed for histopathologic analysis and isolation of poly A+ RNA. CCl4 produced 10-40% liver necrosis in the 6, 24, and 48 hr samples and 0-<10% necrosis in the 72 hr samples. APAP produced 10-40% necrosis at 24 hr, 0-40% at 48 hr, and 0->50% in the 72 hr samples. Mrp3 mRNA expression was significantly increased at 6, 24, and 48 hours following CCl4 administration and at 72 hours after APAP administration. In conclusion, induction of Mrp3 during compensatory hepatocellular proliferation may be important to the enhanced resistance to chemical -induced cytotoxicity in this model. (Supported by NIH grant ES10093).

1777 PROTECTION AGAINST ACETAMINOPHEN HEPATOTOXICITY BY CLOFIBRATE PRETREATMENT : EFFECT OF CATALASE INHIBITION.

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Mice pretreated with the peroxisome proliferator clofibrate (CFB) are highly resistant to acetaminophen (APAP)-induced hepatotoxicity. The objective of the present study was to investigate whether the increase in hepatic catalase activity after CFB pretreatment plays a role in the hepatoprotection. An irreversible catalase inhibitor, 3-amino-1, 2, 4-triazole (3-AT), was used to modulate enzyme activity. Hepatic catalase activity in mice pretreated with CFB (500 mg/kg, i.p. for 10 days) was significantly inhibited by 3-AT (either 0.1 or 0.5 g /kg, i.p.). In addition, the lower dose of 3-AT (0.1 g /kg) had little effects on biliary and urinary excretion of APAP and its metabolites, which suggests that APAP metabolism was not affected by this dose of 3-AT. In toxicity studies, the mortality rate of corn oil-pretreated mice challenged with APAP (800 mg/kg, p.o.) was significantly increased by 3-AT (0.1 g /kg, i.p.) given one h before APAP. As expected, CFB pretreatment conferred full protection against APAP-induced hepatotoxicity. The same 3-AT treatment, however, did not abolish the hepatoprotection in CFB-pretreated mice, despite the marked inhibition of hepatic catalase activity. These results indicate that elevated catalase activity in mice exposed to CFB does not appear to mediate the hepatoprotection. Our findings also provide additional support for the role of reactive oxygen species in APAP-induced hepatotoxicity. (Supported by NIH grant ES10093).

1778 ORGANIC ANION-INDUCED CHOLESTASIS PROTECTS FROM ACETAMINOPHEN (APAP) HEPATOTOXICITY IN MALE CD-1 MICE.

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Previous studies showed that administration of the non-metabolizable organic anion indocyanine green (ICG) immediately prior to a toxic dose of APAP reduces liver injury 24 hours after challenge. ICG produces dose dependent decreases in bile flow (cholestasis) in mice and rats. Studies performed in rats suggest that cholestasis can play a role in this protection. Therefore, this study was conducted to determine if the cholestatic properties of this and other model organic anions is relevant to the protection against APAP liver injury previously observed in mice receiving ICG. For this purpose, overnight fasted male CD-1 mice were dosed i.v. with the cholestatic dyes: bromcresol green (BCG, 30umol/kg) and rose bengal (RB, 60 umol/kg) immediately prior APAP challenge (500mg/kg, i.p.). Other groups of mice received the non-cholestatic dyes dibromosulphthalein (DBSP, 150umol/kg) and amaranth (AM, 300umol/kg) prior to APAP. Controls were given vehicle only. Liver toxicity was assessed 24 hrs later. Hepatocellular necrosis was evident at 24 hrs in control mice receiving APAP, as determined by sorbitol dehydrogenase activity and histopathology. Pretreatment with the cholestatic dyes BCG and RB decreased the severity of hepatocellular necrosis induced by APAP. However, the administration of the non-cholestatic dyes DBSP and AM did not alter APAP-induced liver damage. APAP-induced glutathione depletion was not altered by pretreatment with any of these dyes. Since cholestatic dyes were able to protect from APAP-induced liver damage while non-cholestatic dyes did not, these data indicate that cholestasis is a contributing factor to the changes in susceptibility to APAP hepatotoxicity produced by some of these cholephilic dyes. (Supported by NIH grant ES10093).

1779 MECHANISMS OF METALLOTHIONEIN PROTECTION AGAINST ALCOHOLIC HEPATOTOXICITY: CRITICAL ROLE OF ZINC.

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Metallothionein (MT) is a low molecular weight, cysteine rich (20 cysteinyl residues among 61 amino acids) protein. MT plays an important role in cellular zinc homeostasis. Our previous studies using MT-overexpressing transgenic mice demonstrated that MT is a potent cytoprotective agent against alcoholic liver injury. The present study was undertaken to determine (1) whether MT provides protection against alcoholic liver injury through zinc, and (2) whether zinc prevents alcoholic hepatotoxicity through its antioxidant property. An unique MT-knockout (MT-KO) mouse model was used. MT-KO mice along with their wild-type 129/Sv mice were assigned into four groups: control, ethanol-only, zinc-only and zinc-ethanol. Ethanol was administrated by gavage at a dosage of 5g/kg every 12 h for 3 doses, and zinc sulfate was injected by i.p. at a dosage of 5 mg zinc/kg/day for three days before ethanol treatment. Hepatic MT and zinc concentrations, liver pathological changes and oxidative stress were determined. MT-KO mice showed undetectable hepatic MT content and lower zinc concentration than wild-type mice. Zinc treatment significantly elevated hepatic MT as well as zinc concentrations in wild-type mice and only hepatic zinc concentrations in MT-KO mice. Under light and electron microscopes, pathological changes were observed in the livers treated with ethanol, while the cellular injury was much severer in the MT-KO mice. However, this acute alcoholic hepatotoxicity was significantly inhibited by zinc in both MT-KO and wild-type mice. Furthermore, ethanol treatment decreased hepatic reduced glutathione concentration and increased hepatic lipid peroxidation, though wild-type mice showed less lipid peroxidation than MT-KO mice. This hepatic oxidative stress was abrogated by zinc pretreatment in both MT-KO and wild-type mice. These results thus demonstrate that zinc plays a critical role in MT protection against alcoholic liver injury through its antioxidant action.

1780 METALLOTHIONEIN-NULL MICE ARE SENSITIVE TO ENDOTOXIN/D-GALACTOSAMINE-INDUCED HEPATOTOXICITY.

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Metallothionein (MT) is induced during acute-phase. It is thought to regulate zinc and copper homeostasis, detoxify heavy metals and scavenge free radicals. However, the specific function of MT in the acute-phase response remains to be elucidated.

We examined whether MT acts as a protective factor or not. Mice treated with endotoxin (LPS)/D-galactosamine (GalN) selectively develop hepatic failure. We found that MT-null mice were more sensitive to LPS/GalN-induced lethality than wild-type mice. The increase of serum TNF-α, a vital mediator, in MT-null mice was similar to wild-type mice. To investigate whether intracellular MT can directly inhibit TNF-induced hepatocyte death, we compared sensitivity to TNF/actinomycinD (ActD)- and TNF/Ad5IκB, an inhibitor of IκB degradation, - induced cytotoxicity. The degree of cytotoxicity caused by TNF/ActD and TNF/Ad5IκB in primary hepatocytes from wild- type mice was similar to MT-null mice. Hepatocyte apoptosis precedes liver injury in mice administered with LPS/GalN. Hepatic apoptosis caused by LPS/GalN was detected only in MT-null mice under our experimental conditions. The induction of hepatic α_1-acid glycoprotein (AGP) mRNA in the response to LPS/GalN was decreased in MT-null mice compared to that in wild type mice. The acute-phase protein AGP has been demonstrated to protect mice from LPS/GalN-induced hepatic apoptosis and hepatotoxicity. These results suggest that MT might have the potential to prevent LPS/GalN-induced hepatotoxicity, at least, through the enhancement of AGP induction.

1781 CASPASE ACTIVATION AND CYTOCHROME C RELEASE IN RAT LIVER FOLLOWING TREATMENT WITH GALACTOSAMINE.

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Galactosamine is a well-known model hepatotoxin. The hepatic lesion described by Keppler et al. in 1968 is a scattered focal hepatic necrosis. Recent reports have demonstrated that galactosamine also induces hepatocyte apoptosis when given to rats or mice. In the present studies we wished to determine which cell signaling pathways might contribute to the development of hepatocyte apoptosis following galactosamine treatment in rats. Studies by Stachlewitz et al. (1999) suggested that galactosamine-induced hepatocyte apoptosis may be mediated in part through activation of Kupffer cells and their production of TNFα. To distinguish between TNFα-mediated and non-TNFα mediated apoptosis pathways, we determined the activation of caspases 8 and 9 and the release of cytochrome c from the mitochondria. Caspase activities were assessed in liver cytosolic fractions at various times (1-12 hours) following galactosamine treatment of rats. Activation of caspases 8 and 9 was determined by cleavage of the substrates IET-pNA or LEHD-pNA, respectively. Our data suggest that caspase 8 activation occurs within 1 hour following galactosamine treatment. Caspase 9 activation was not observed until 6 hours following galactosamine treatment. At the peak time of apoptosis, 12 hours after treatment, cytochrome c release from mitochondria was clearly detected in livers of rats treated with galactosamine. Cytochrome c was not detected in the cytosolic fraction of livers from control rats. These data suggest that following galactosamine treatment, the TNFα-mediated apoptosis pathway (caspase 8 activation) may be activated in hepatocytes initially, followed by activation of the mitochondrial apoptosis pathway (cytochrome c release and caspase 9 activation). A thorough determination of the timecourse of caspase activations and cytochrome c release will confirm the importance and contributions of the TNFα-mediated pathway *versus* cytotoxic damage to the development of galactosamine-induced hepatocyte apoptosis.

1782 BACTERIAL LIPOPOLYSACCHARIDE EXPOSURE ALTERS AFLATOXIN B_1 HEPATOTOXICITY: BENCHMARK DOSE ANALYSIS FOR MARKERS OF LIVER INJURY.

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Aflatoxin B_1 (AFB_1) is a toxin produced by fungi that causes both acute hepatotoxicity and hepatocellular carcinoma in humans and experimental animals. Epidemiological studies have shown that individuals exposed to AFB_1 who have concurrent hepatitis B infection, a disease characterized by chronic inflammation, are at greater risk of developing liver carcinoma. Previous work from this laboratory demonstrated that a small, noninjurious dose of bacterial lipopolysaccharide (LPS) augments the hepatotoxicity of AFB_1 through activation of inflammatory cells resulting in the production of soluble inflammatory mediators. This study was conducted to examine the effect of a noninjurious dose of LPS on the dose-response relationship for AFB_1-induced liver injury. Male, Sprague-Dawley rats (250-350g) were treated with AFB_1 (0.1 mg/kg-6.3 mg/kg ip) and 4 hours later with *E. coli* LPS (7.4×10^6EU/kg, iv). Twenty-four hours after AFB_1 administration, hepatic parenchymal cell injury was determined from elevations in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. Injury to intrahepatic bile ducts was evaluated from increased serum γ-glutamyl transferase and alkaline phosphatase activities. Based on benchmark dose (BMD) analysis, the AFB_1 BMD for parenchymal cell injury was decreased 10-fold by LPS cotreatment, whereas AFB_1 BMDs for bile duct injury were decreased nearly 20-fold. Overall, the data suggest that concurrent inflammation renders the liver considerably more sensitive to the hepatotoxic effects of AFB_1. (Research supported by NIH grant ES04139)

1783 MECHANISMS OF POTENTIATION OF CARBON TETRACHLORIDE HEPATOTOXICITY AND LETHALITY IN TYPE II (NIDDM) DIABETIC RATS.

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The objective of this study was to develop a refined animal model of Type II diabetes and investigate the effect of CCl_4 hepatotoxicity in Type II diabetic rats. Diabetes was induced in male Sprague-Dawley rats (100-124 g) maintained on high fat diet (20% fat, 24% protein, 54% carbohydrate) for 24 days. On day 14, streptozotocin (STZ, 45 mg/kg, ip, in citrate buffer) was injected. Appropriate controls i.e. high fat diet, normal diet and normal diet + STZ were maintained. On day 24, Type II diabetes was confirmed by hyperglycemia (600 ± 13 mg/dl) and normoinsulinemia before use in experiments. An ordinarily nonlethal dose of CCl_4 (2 ml/kg, ip, 1:1 in corn oil) yielded 100% mortality in Type II diabetic rats. A time course study of 0 to 96 h was conducted after CCl_4 challenge. In the diabetic rats, liver injury increased as assessed by plasma ALT, AST and histopathology. Liver function, measured by plasma bilirubin, was markedly decreased at 24 h. Liver glycogen measured as an indicator of hepatic energy status declined. Death resulted due to hepatic failure as indicated by hyperammonemia and hypoglycemia between 24 to 48 h. Hepatic microsomal CYP 2E1 measured by Western blot analysis was induced in Type II diabetic rats, about 40% above controls. Tissue repair, assessed by ^{3}H-thymidine incorporation and proliferating cell nuclear antigen assay (PCNA), was inhibited. In the non-diabetic rats liver injury peaked at 48 h but timely and robust tissue repair resulted in recovery. In diabetic rats this dose resulted in liver injury that peaked at 24 h and insufficient tissue repair led to progression of injury and animal death. In summary, CCl_4 hepatotoxicity is potentiated due to a combination of higher bioactivation following induction of CYP 2E1 and compromised tissue repair in Type II diabetic rats. These findings suggest that just as Type I diabetes, Type II diabetes also is a predisposition factor for CCl_4 hepatotoxicity.

1784 PROTECTIVE MECHANISMS AGAINST ACETAMINOPHEN TOXICITY IN DIABETIC MICE: ROLE OF PPAR-α ACTIVATION.

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Our recent work has demonstrated diabetic mice to exhibit significantly less liver injury and mortality together with augmented compensatory tissue repair after challenge with acetaminophen (APAP). Diabetes causes activation of PPAR-α just as peroxisome proliferators, and both protect against APAP toxicity. The present work examines the mechanistic role of PPAR-α activation in diabetes as a hepatoprotective mechanism against lethal APAP challenge. PPAR-$\alpha^{-/-}$ mice made diabetic by streptozotocin (150 mg/kg, ip, citrate buffer) did not show CYP4A induction (a marker of PPAR-α activation), unlike wild-type (WT) diabetic phenotypes which showed considerable CYP4A induction. On treatment with APAP (600 mg/kg, ip in basic saline, a LD_{100} dose in WT non-diabetic mice), WT-diabetic mice showed only 30% mortality and 50% less liver injury measured by ALT and histopathology. In contrast, diabetic-PPAR-$\alpha^{-/-}$ mice did not exhibit any protection against APAP toxicity suggesting the importance of PPAR-α in diabetes-induced protection. S-phase DNA synthesis and PCNA histochemical staining after injury showed earlier and robust tissue repair in WT-diabetic mice, but not in the PPAR-$\alpha^{-/-}$diabetic mice. The upregulation of compensatory tissue repair in the diabetic mice appears to be mediated *via* PPAR-α activation. Although hepatic CYP2E1 and 1A2, which are involved in APAP bioactivation, were lower in the WT-diabetic mice, covalent binding of APAP was unchanged. Western analysis of APAP bound to 58, 56 and 44 KDa acetaminophen binding proteins was similar among diabetic and non-diabetic mice. Here we report PPAR-α activation and upregulated liver tissue repair as primary mechanisms of protection against APAP toxicity in diabetes.

1785 UPREGULATED SIGNAL TRANSDUCTION ENHANCES LIVER TISSUE REPAIR UPON TOXIC CHALLENGE IN CALORIC RESTRICTED RATS.

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We have previously reported that moderately caloric or diet restricted (DR) rats are protected from lethal hepatotoxic challenge of thioacetamide [600 mg/kg TA; 90% lethality in Ad libitum (AL) vs 30% in DR rats] due to timely and robust compen-

satory liver tissue repair response. This study was designed to investigate the role of various signal transduction pathways in the stimulation of higher liver regeneration in the DR rats over a time course of 0 to 72 h after TA administration. Pro-inflammatory cytokines TNF-α and IL-6 were estimated in plasma by ELISA and in liver by either RT-PCR analysis or immunohistochemistry. Although, no significant difference in plasma TNF-α levels was observed between AL and DR, slightly higher TNF-α mRNA was found in the livers of DR rats at 48 and 72 h after TA injection. Plasma IL-6 was significantly higher in DR, which peaked at 48 h (6-fold higher than in AL). IL-6 in liver measured by immunohistochemical analysis was consistent with these results. TNFR-I and IL-6 R mRNA did not change in AL or DR at any time after TA administration. Expression of growth factors TGF-α and HGF was studied by immunohistochemical analysis in livers of AL and DR rats after TA administration. DR rats exhibited higher and consistent expression of both TGF-α and HGF during the phase of active mitogenesis while expression in AL rats was decreased and diminished. Western blot analysis of EGFR (TGF-α receptor) indicated that DR rats have initial higher EGFR protein levels which remained consistently higher until 48 h. Similar higher c-met (HGF receptor) protein was observed in DR rats at 48 h. Taken together, these data indicate that the higher liver tissue repair is stimulated in DR rats by timely expression of the pro-mitogenic factors leading to subsequent upregulation of the signal transduction pathways involved in liver regeneration. (Supported by ES-09870)

1786 HIGHER EXPRESSION OF TNF-α AND IL-6 IN LIVER OF DIET RESTRICTED RATS AFTER THIOACETAMIDE-INDUCED LIVER INJURY.

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Previous studies in our laboratory have shown that moderate diet restriction (DR, 35% for 21 days) protects male Sprague-Dawley rats from lethal dose of thioacetamide (TA). The protection in DR rats is due to enhanced liver tissue repair. Studies indicate that proinflammatory cytokines, TNF-α, IL-6 and their receptors, may play an important role in stimulating quiescent liver cells to proliferate. The present studies were designed to investigate TNF-α/TNFR1 and IL-6/IL-6R expression in TA poisoned rats over a time course of 0 to 72h after injecting TA. AL and DR rats received an ordinarily lethal dose of TA (600 mg/kg in saline, ip). Liver injury was measured by ALT, AST and histopathology, and tissue repair was measured by ^{3}H-thymidine incorporation and PCNA. Plasma TNF-α and IL-6 were assayed by ELISA. Expression of mRNA in the liver was detected by semi-quantitative RT-PCR. The data indicate that TNF-α in plasma and its mRNA in liver tissue increase in both DR and AL groups after TA administration. There was no significant difference in plasma TNF-α in DR and AL rats at any time point. DR rats had significantly higher TNF-α mRNA in liver at 24 to 72h as compared to AL rats. IL-6 in plasma and its mRNA in liver also increased after TA administration. DR rats had 6-fold higher plasma IL-6 at 48h coincidental with higher IL-6 mRNA in the liver. Immunohistochemical analysis of IL-6 in liver indicated localization mainly in sinusoidal endothelial cells, suggesting that these may be the source of IL-6. In both AL and DR rats, TNFR1 and IL-6R mRNA expression was similar over the time course. Because expression of TNFR1 and IL-6R was unchanged, upregulation of pro-mitogenic signaling is achieved by up-regulated TNF-α and IL-6 in DR rats compared with AL rats. These results indicate that higher expression of TNF-α and IL-6 may play an important role in higher tissue repair in DR rats upon liver injury induced by TA. (Supported by ES-09870)

1787 EFFECTS OF TICLOPIDINE, AN ANTIPLATELET AGENT, ON RAT MODELS OF HYPERBILIRUBINEMIA.

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Ticlopidine, an anitplatelet agent, has been reported to cause cholestatic hepatitis in patients with rare cases. To approach the mechanism underlying the event, we investigated effects of ticlopidine on hepatic function and morphology of Eisai hyperbilirubinuria rats (EHBR), Sprague-Dawley rats (SDR), as an original strain of EHBR, and bile duct ligated rats. Repeated oral administration of ticlopidine (100 and 300 mg/kg/day for 7 consecutive days) remarkably raised serum bilirubin level only in EHBR from day 2. On day 7, the bilirubin level was 5-8 times higher (control 1.37 mg/dL vs 7.41-11.24 mg/dL) than that of the vehicle control. This change was accompanied by slight elevations in serum bile acid, cholesterol and phospholipid levels without increased serum transaminase activities. Histopathologically, no hepatic necrosis and cholestasis were enhanced. In SDR and bile duct ligated rats, deterioration was neither functionally nor morphologically observed. To elucidate the mechanism of ticlopidine-enhanced hyperbilirubinemia in EHBR, the effects on bile flow and, bilirubin and bile acid excretion into the bile were investigated. Ticlopidine did not affect these parameters, suggesting that impairment of biliary excretion did not relate to the enhanced serum bilirubin level. In the analysis of hepatic transporters mRNA levels involved in bilirubin and bile acid transport by a real time PCR, a significant increase in rat multidrug resistance-associated protein 3 (Mrp3) mRNA level as a transporter for excretion from the liver into peripheral blood was noted. Messenger RNA level of heme oxygenase-1, a rate-limiting enzyme in the catabolism of heme to bilirubin, was also elevated compared to the vehicle control ($P<0.05$ by StudentÅfs t test), whereas this phenomenon was not seen in SDR. These results provide the possibility that the facilitation of bilirubin formation and transport from the liver into peripheral blood through Mrp3 may contribute to ticlopidine-enhanced hyperbilirubinemia in EHBR.

1788 QUANTIFICATION OF HEPATOCYTE PROLIFERATION AND APOPTOSIS FOLLOWING ILEAL RESECTION IN RATS.

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Bile acids regulate the expression of various molecules involved in cholesterol and lipid metabolism by binding the nuclear receptor FXR and activating FXR-mediated transcription. Evidence also suggests that bile acids may regulate the proliferation of hepatocytes. To investigate the role of bile acids on hepatocyte proliferation and apoptosis *in vivo*, we used a rat model of ileal resection to reduce the ileal bile acid transporter (IBAT)-mediated enterohepatic recirculation of bile acids. Rats were sacrificed 2, 4, and 13 weeks following ileal resection. Cholesterol 7alpha-hydroxylase (Cyp7a) activity and fecal bile acid concentrations were determined to demonstrate the decrease in ileal bile acid reabsorption. Proliferating and apoptotic hepatocytes were detected by immunohistochemistry using PCNA as a marker of cell proliferation and activated caspase-3 as a marker of apoptosis. The proliferation and apoptotic rates were quantified using computer-assisted image analysis. Our results demonstrated a slight decrease in liver weights and significant increases in Cyp7a activity and fecal bile acid concentrations following ileal resection. In addition, a moderate, but significant decrease in hepatocyte proliferation was observed 2 and 4 weeks after surgery, while no differences in the apoptotic rate among groups were detected at any time points. In conclusion, inhibition of ileal bile acid reabsorption through surgical ileal resection is associated with slight decreases in the rate of hepatocyte proliferation and slightly decreased liver weight, suggesting that physiologic concentrations of bile acids may regulate hepatocyte proliferation *in vivo*.

1789 INTERACTIONS OF COMBINED BILE ACIDS ON HEPATOCYTE VIABILITY: CYTOPROTECTION OR SYNERGISM.

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Cholestasis results from hepatocyte dysfunction due to the accumulation of bile acids in the cell, many of which are known to be cytotoxic. Recent evidence suggests that through competing reactions nontoxic bile acids might afford cytoprotection against cholestasis. In this work, we compare the relative cytotoxicity of bile acids based on a dose- and time-dependent study. To better elucidate the controversy related to the therapeutic use of ursodeoxycholate (UDCA) in cholestatic patients, we also evaluated the effects of bile acid combinations. Viability of Wistar rat hepatocytes in primary culture was measured by LDH leakage after 12 and 24 hr exposure of cells to the various bile acids. All unconjugated bile acids caused a dose-dependent decrease in cell viability. The tauro- and glyco-conjugates of chenodeoxycholate (CDCA) and UDCA were all less toxic than the corresponding unconjugated form. Although relatively non-toxic, UDCA caused synergistic cell killing by lithocholate (LCA), CDCA, glyco-CDCA (GCDC) and tauro-CDCA (TCDC). Glycoursodeoxycholate decreased the toxicity of GCDC but potentiated the toxicity of unconjugated CDCA and LCA. The tauro-conjugate of UDCA had no significative effect. These data suggest that at cholestatic relevant concentrations, bile acid-induced cell death is dependent on the degree of lipophilicity and conjugative status of individual bile acids. However, these results indicate that the reported improvement of biochemical parameters in cholestatic patients treated with UDCA is not due to a direct effect of UDCA on hepatocyte viability. Therefore, any therapeutic effect of UDCA must be secondary to an altered membrane transport or nonparenchymal cell function.

1790 EFFECTS OF BETAINE ON HEPATIC FIBROSIS INDUCED BY NITROSODIMETHYLAMINE IN RATS.

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Effects of betaine intake on hepatic fibrogenesis were examined in adult male SD rats treated with nitrosodimethylamine (DMN; 10 μl/kg/day, ip) 3 days a week for 4 weeks. From two weeks prior to initiation of DMN treatment until sacrifice rats

were provided with drinking water containing 1 % betaine. DMN treatment at this dosage regimen resulted in hepatic fibrosis as determined by changes in hepatic 4-hydroxyproline contents and serum biochemical parameters, such as alanine aminotransferase, aspartate aminotransferase, total bilirubin and plasma protein contents. Hepatic microsomal enzyme activities were also reduced in rats treated with DMN. Betaine intake significantly decreased the changes induced by DMN, which was further supported by the results of histopathological examination of rat liver. DMN treatment also elevated hepatic concentration of malondialdehyde measured by HPLC analysis, which was completely blocked by betaine. In liver of rats treated with DMN, S-adenosylmethionine level was decreased but cysteine, taurine, glutathione disulfide and homocysteine levels were all increased. Consistent changes in the activities of critical enzymes involved in the transsulfuration pathway were also observed. Betaine intake inhibited all the changes in the metabolic intermediates/products and enzyme activities induced by DMN. It is suggested that antifibrotic activity of betaine could be associated with its potential for maintaining homeostasis of hepatic sulfur-containing amino acid metabolism.

1791 ENDOTHELIAL CELL INJURY AND FIBRIN DEPOSITION IN RAT LIVER AFTER MONOCROTALINE EXPOSURE.

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Monocrotaline (MCT) is a pyrrolizidine alkaloid plant toxin that produces hepatotoxicity in people and animals. Human exposure to MCT occurs through consumption of contaminated grains and herbal medicines. Intraperitoneal injection of 300 mg/kg MCT into rats produced time-dependent hepatic parenchymal cell (HPC) toxicity beginning 12 hours after treatment. To determine the development of damage to sinusoidal endothelial cells (SECs) in the liver, SEC injury was quantified using immunohistochemical staining and by increased plasma hyaluronic acid concentration. MCT produced extensive SEC injury that was restricted to centrilobular regions by 8 hours after treatment. To determine if damage to SECs in the liver stimulated activation of the coagulation system, fibrin deposition was quantified in the liver using immunohistochemistry. Extensive fibrin deposition occurred in the liver after MCT treatment and was restricted to centrilobular regions. Fibrin deposition occurred simultaneously with SEC damage and before HPC injury. Accordingly, the hypothesis was tested that activation of the coagulation system is required for MCT-induced liver injury. Treatment of rats with anticoagulant heparin significantly prevented MCT-induced fibrin deposition in the liver and HPC injury. By contrast, heparin did not prevent MCT-induced damage to SECs. These results suggest that activation of the coagulation system is required for MCT-induced HPC injury but not SEC injury in the liver. (Supported by NIH grants ES04139 and DK50728.)

1792 PROGRESSIVE HEPATOTOXICITY DUE TO A THIAZOLIDINEDIONE CONTAINING ANALOGUE OF N-(3, 5-DICHLOROPHENYL)SUCCINIMIDE (NDPS) IN FISCHER 344 RATS.

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3-(3', 5'-dichlorophenyl)thiazolidine-2, 4-dione (DCPT) is an analogue of the nephrotoxic agricultural fungicide, N-(3, 5-dichlorophenyl)succinimide (NDPS). The target organ for toxicity changed from kidney to liver when the succinimide ring of NDPS was replaced by the thiazolidinedione (TZD) ring of DCPT. To further investigate DCPT-induced toxicity a time course study was conducted. Male, Fischer 344 rats were administered DCPT (0.6 mmol/kg, i.p. in corn oil), and kidney and liver function were assessed at 24, 72, and 96 hours post-dosing. DCPT treatment produced marked and persistent diuresis. Ketonuria was seen up to day 2 of the experiment. A sharp increase in urine glucose levels was observed from day 1 to day 2. However, by day 4 urine glucose values returned to day 0 levels. Also, body weight, and water and food consumption decreased on day 1, but were elevated above day 0 values by day 2 and beyond. Kidney and liver weights were not elevated throughout the experiment, however alanine aminotransferase (ALT) levels were increased at 24 hours (380.00 ± 63.77), compared to 72 (25.75 ± 4.80) and 96 (25.25 ± 3.33) hours post-dosing. The histological data support these toxicological findings because there was a focal infiltration of neutrophils from the vasculature into the surrounding tissue 24 hours post-dosing. At 72 and 96 hours the neutrophils were absent, however diffuse damage was seen throughout the liver. The results suggest that DCPT has diuretic, ketonuric and glucosuric activity. Furthermore, DCPT appears to produce progressive hepatotoxicity in rats. Since liver damage was reported in patients taking troglitazone and other TZD-containing insulin sensitizers, DCPT may be a useful model compound for studying the toxicity of these drugs. However, the role of the TZD ring in DCPT-induced hepatotoxicity requires further investigation.

1793 LACK OF SEX DIFFERENCES IN ALCOHOL-INDUCED LIVER DAMAGE IN RATS FED LOW CARBOHYDRATE DIETS.

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It has been reported that female rats are significantly more susceptible to alcohol-induced liver damage (ALD) than males, in a model of ALD which utilizes intragastric feeding (Iimuro et al. Am. J. Physiol. 272:G1186-94, 1997). This has been linked to estrogen-associated increases in Kupffer cell expression of the CD14 endotoxin receptor. We have attempted to replicate these data using two different models of ALD, one involving intragastric feeding of ethanol as part of total enteral nutrition (TEN) and one involving the use of a low carbohydrate liquid diet. Both of these models have previously been shown to produce ALD, but do not result in statistically significant increases in plasma endotoxin. Groups of N = 8 to 10 male and female Sprague-Dawley rats, 300 g were placed on the TEN system or the liquid diets for 42 to 60 days. Blinded pathological evaluation of liver samples was performed. In both models, ethanol treatment at 12 to 13 g/kg/d produced statistically significant liver hyperplasia accompanied by increases in plasma ALT, steatosis, inflammation and necrosis and induction of CYP2E1 ($P \leq 0.05$). However, in neither model, were females significantly more damaged than males. Total pathology score: TEN EtOH males 10 ± 0.6 and females 8.7 ± 0.6; liquid diet model EtOH males 7.7 ± 0.7 and females 8.1 ± 0.5. Thus, our data do not support the previous conclusion that female rats have a greater susceptibility to ethanol-induced hepatotoxicity than males. Supported in part by R21 AA12031 (T.M.B).

1794 EFFECTS OF THE DIETARY ANTIOXIDANTS BUTYLATED HYDROXYTOLUENE (BHT) AND N-ACETYL CYSTEINE (NAC) ON ALCOHOL-INDUCED LIVER DAMAGE IN A RAT MODEL UTILIZING TOTAL ENTERAL NUTRITION.

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We have developed a total enteral nutrition (TEN) model to study alcohol-induced liver damage (ALD). It has been suggested that development of ALD is dependent upon free radical formation as the result of CYP2E1-dependent metabolism of ethanol and unsaturated fatty acids. In this study we examined the protective effects of two different antioxidants – the free radical scavenger BHT and the glutathione precursor NAC. Six groups of N = 8-10 Sprague-Dawley rats (300 g)were used. Groups were placed on TEN, TEN + BHT (150 mg/kg/d), TEN + NAC (4.2 g/kg/d), EtOH, EtOH + BHT and EtOH + NAC for 45 d. NAC treatment significantly increased total anti-oxidant capacity of liver cytosol in TEN controls using the ORAC assay ($P \leq 0.05$). Ethanol treatment reduced ORAC values significantly in both the EtOH and EtOH + NAC groups ($P \leq 0.05$) but the values for the EtOH + NAC group were insignificantly different from TEN controls showing that NAC consumption significantly protected the liver from oxidative stress produced by EtOH. In contrast BHT treatment reduced ORAC values in control animals but EtOH treatment had no further effect. Whereas CYP2E1 apoprotein was induced 14-fold in EtOH and EtOH + NAC groups, it was only induced 5-fold in the EtOH + BHT group. Significantly elevated liver weight, plasma ALT ($P \leq 0.05$) and typical pathology associated with ALD (steatosis, inflammation and necrosis) were observed in all three groups of ethanol-treated rats and neither treatment provided significant protection against ethanol-associated liver damage. These data cast doubt upon the roles of CYP2E1 and free radicals in development of ALD. Supported in part by AA08645 (T.M.B).

1795 POTENTIATION OF ALLYL ALCOHOL HEPATOTOXICITY BY ENDOTOXIN REQUIRES ACTIVATION OF THE COAGULATION SYSTEM.

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Noninjurious doses of bacterial endotoxin (LPS) potentiate allyl alcohol-induced liver damage in rats. This enhanced injury could not be replicated in the isolated, buffer-perfused liver and was attenuated *in vivo* by heparin, suggesting that the coagulation system is required for the response. Inasmuch as heparin has effects other than anticoagulation, rats were dosed with warfarin (20 mg/kg, p.o.) or its vehicle

42 and 18 hours before LPS ($1.2x10^6$ EU/kg, i.v.). Rats were then treated two hours after LPS with allyl alcohol (30 mg/kg, i.p.). Like heparin, warfarin attenuated the increase in serum ALT activity of rats treated with LPS/allyl alcohol, suggesting that the coagulation system contributes to hepatic damage in this model. Thrombin is a component of the coagulation cascade that is inhibited by both heparin and warfarin. To assess the specific role of thrombin in the injury, livers were isolated from rats treated 2 hours earlier with LPS ($1.2x10^6$ EU/kg, i.v.) or sterile saline and were perfused with buffer containing thrombin (70 units/liver) or vehicle and various concentrations of allyl alcohol. Thrombin did not increase the sensitivity of the livers to allyl alcohol. This result suggests that thrombin does not contribute to hepatic damage through activation of receptors in the liver. Allyl alcohol-induced toxicity was greater in isolated, perfused livers from LPS-pretreated compared to vehicle-pretreated rats, indicating that some change occurs within the first 2 hours after LPS exposure to increase the sensitivity of the liver to allyl alcohol. This change may be related to accumulation of neutrophils and other blood components in the liver. In summary, LPS augments the hepatotoxicity of allyl alcohol through a mechanism involving the coagulation cascade, and the role of thrombin may be dependent on other blood components (supported by ES08789).

1796 ACTIVATION OF PROTEIN KINASE C δIN ALLYL ALCOHOL-INDUCED TOXICITY IN RAT HEPATOCYTES.

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Allyl alcohol (AA)-induced toxicity in hepatocytes is well established, though the specific mechanism by which AA leads to cell death is not known. Several downstream effects of AA have been linked to cell killing, including protein sulfhydryl loss and lipid peroxidation. However, early events in the process have not been identified. The purpose of these studies was to examine whether specific signal transduction pathways could be associated with AA toxicity in hepatocytes. Rat hepatocytes were isolated, adhered to tissue culture dishes and used for experiments within 3 hours of plating. Inhibitors of various signal transduction pathways were added to cells prior to AA addition, and alanine aminotransferase (ALT) release was measured as an indicator of hepatocyte injury/death. Inhibition or augmentation of cAMP/protein kinase (PK) A by Rp-cAMPS or IBMX, respectively, had no effect on AA-induced cell death. H-7, an inhibitor of PKA, PKC and PKG, partially inhibited cell killing by AA. Whereas, chelerythrine chloride, a panspecific PKC inhibitor, almost completely abrogated AA cytotoxicity. Use of selective inhibitors of PKC isoforms showed no effect on cell killing by HBDDE, a PKC αand β inhibitor, or by bisindolylamide I, an inhibitor of PKC α, β, and ε. In contrast, rottlerin, a PKC δ inhibitor, blocked hepatocellular killing by AA. Experiments with chelerythrine chloride and rottlerin were repeated using acrolein instead of AA to test whether the PKC inhibitors were preventing the bioactivation of AA to acrolein, and results again showed marked inhibition of ALT release by chelerythrine chloride and rottlerin. Upon activation, PKC δ is phosphorylated and translocates from the cytosol to cell membranes. Western blotting and immunohistochemistry techniques revealed phosphorylation and translocation of PKC δ to hepatocyte membranes by AA within 30 minutes. Together, these results indicate that activation of PKC δ is a critical early event in initiating hepatocyte injury and death by AA. (Supported by NIEHS 08789)

1797 *IN VITRO* METABOLISM OF DIETHYLENE GLYCOL MONOETHYL ETHER (DEGEE) AND ETHYLENE GLYCOL MONOETHYL ETHER (EGEE) BY RAT AND HUMAN HEPATOCYTES.

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Ethylene glycol monoethyl ether (EGEE) is converted to a toxic metabolite, ethyoxyacetic acid (EAA). Diethylene glycol monoethyl ether (DEGEE) is similar structurally to EGEE but its metabolism is not known. The objective of this study was to compare the *in vitro* metabolism of DEGEE and EGEE by rat and human hepatocytes to determine whether DEGEE is likely to be converted to toxic metabolites *in vivo*. Rat and human hepatocytes were isolated and suspensions were incubated with 15, 150, and 1500 μM ^{14}C-DEGEE or 150 μM ^{14}C-EGEE. Aliquots of the incubations were removed up to 4 hr and were analyzed by HPLC with radiochemical detection. At the end of the 4 hr incubation period, over 88% of the radioactivity was associated with DEGEE in rat hepatocyte incubations with 150 and 1500 μM ^{14}C-DEGEE and 70% of the radioactivity was ^{14}C-DEGEE in cells incubated with the lowest concentration (15 μM ^{14}C-DEGEE). The rest of the radioactivity was in small, unidentified peaks. Rat hepatocytes converted ^{14}C-EGEE (150 μM) into ethylene glycol (9.65%)and EAA (52.18%). Human hepatocytes did not metabolize ^{14}C-DEGEE to a significant extent; approximately 98 to 99% of the radioactivity remained unchanged. EGEE was readily metabolized by human hepatocytes to ethylene glycol (7.66% and 25.37% for 2 specimens) and EAA (17.57% and 69.50%). In summary, EGEE was readily metabolized by both rat and human hepatocytes to EAA and ethylene glycol, and the rat liver cells metabolized EGEE at a higher rate than human liver cells, in agreement with previously published *in vitro* metabolism data (Green et al., Occup. Hyg., **2**, 67-75, 1996). In contrast, DEGEE was slowly metabolized by rat hepatocytes to several different unidentified metabolite peaks that accounted for as much as 17% of the total radioactivity. Human hepatocytes did not metabolize DEGEE significantly. No EAA or EGEE was formed from DEGEE by either rat or human hepatocytes.

1798 SPECIES COMPARISON OF VITAMIN K1 2, 3-EPOXIDE REDUCTASE ACTIVITY.

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In the hepatic vitamin K cycle, vitamin K1 2, 3-epoxide reductase is the microsomal enzyme responsible for catalyzing the reduction of vitamin K1 2, 3-epoxide (VKO) to vitamin K1 hydroquinone (VKH2). VKH2 is a required cofactor for posttranslational carboxylation of proteins carried out by the carboxylase. This modification is essential for synthesis of biologically active factors II (prothrombin), VII, IX, and X, and proteins C, S, and Z of the blood coagulation system. To expand our understanding of hepatic vitamin K metabolism and function across species, a comparative study of vitamin K1 2, 3-epoxide reductase (VKOR) activity was conducted. Using an *in vitro* assay for VKOR activity, the enzyme kinetic constants Km and Vmax have been determined using bovine (n = 5), canine (n = 5), human (n = 1), murine (n = 5), ovine (n = 5), porcine (n = 5), and rat (n = 5) hepatic microsomes. Microsomal incubations contained 1 ml of microsomes (3 mg protein/ml), the substrate VKO (1 uM - 100 uM), and 2 mM dithiothreitol (reducing agent). Using HPLC/PDA to measure reduced metabolites of VKO formed at each concentration of epoxide, the kinetic constants Km and Vmax were assessed for each species. Tukey's test for multiple comparisons revealed statistically significant species differences ($p<0.0001$) in the Km and Vmax of the enzyme for the epoxide. The greatest statistically significant difference in the Km was observed between murine and rat microsomes, 3.99 ± 0.78 and 10.51 ± 1.66 uM vitamin K1 2, 3-epoxide respectively. Ovine and porcine microsomes had the largest statistically significant difference in the maximum reaction velocity (Vmax) of the enzyme, 1.62 ± 0.07 and 5.23 ± 0.28 umoles vitamin K1 quinone/mg protein/minute respectively. Since VKOR has been shown to be the rate-limiting step in the carboxylation system (Dr. Wallin, personal communication), the data suggest that the posttranslational capacity of the system to produce vitamin K-dependent proteins differs between species.

1799 DIFFERENTIAL ESTRADIOL (E2) AND DIMETHYLBENZANTHRACENE (DMBA) METABOLISM BY MONKEY LIVER MICROSOMES IN RELATION TO TCDD EXPOSURE AND ENDOMETRIOSIS (EM).

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Recently, Rier, et al.(2000) showed that serum E2 levels decreased ($p<0.02$) with TCDD (2, 3, 7, 8- tetrachlorodibenzo-*p*-dioxin) exposure (0 vs 5+25 ppt in diet)in rhesus monkeys (ovariectomized; E2/progesterone replacement) with and without EM. This study examined whether this could be explained by increased liver E2 metabolism, since TCDD induces liver cytochrome P450 enzymes (CYPs) that metabolize E2. DMBA metabolism was also investigated since it is metabolized by similar CYPs. E2 and DMBA metabolism were compared using liver microsomes from the monkeys studied by Rier. Inconsistent with the serum E2 levels, the rate of E2 utilization and the production of E2 metabolites by liver microsomes was generally unaffected by TCDD exposure, except for estriol (E3)production which showed a decrease ($p=0.01$) with TCDD dose. Instead, E2 metabolism was more affected by EM status with decreased production of 2OH-E2 ($p=0.004$), 4OH-E2 ($p=0.003$), and E3($p=0.05$) in monkeys with EM. In animals without EM, the DMBA utilized ($p=0.008$) and the production of the 5, 6-($p=0.04$), 8, 9-($p=0.04$), and 3, 4-($p=0.03$) diols correlated with 2OH-E2 production, and the formation of the 3, 4-($p=0.04$) and 10, 11-($p=0.02$) diols correlated with 4OH-E2 production. In animals with EM, 10, 11-diol formation correlated with estrone production ($p=0.02$), as well as with CYP1A1 (ethoxyresorufin-O-deethylase) activity ($p=0.005$) and protein ($p=0.007$). In this group, decreased serum E2 levels weakly correlated ($p=0.05$) with increased rates of E2 utilization and generally related to increased CYP1A1 (not seen in disease-free animals). The results of this study suggest that the decreased serum E2 levels observed in TCDD-exposed rhesus monkeys with and without EM do not result from a TCDD-induced increase in liver E2 metabolism, but suggest that E2 and DMBA metabolism are differentially altered with disease status (supported by Endometriosis Association, Farleigh Dickinson Foundation, and NIEHS grant ES08539-01).

1800 PROTEIN EXPRESSION CHANGES IN THE RAT LIVER AFTER 90-DAY JP-8 EXPOSURE.

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The military jet fuel JP-8 and its commercial counterpart, Jet A, are used worldwide, causing exposure to liquid fuel, aerosol, and vapor. While most studies have found little toxicity from acute exposure, long-term effects are not well studied. Sprague-Dawley rats were exposed to 250, 500, or 1000 mg/m3 JP-8 vapor for 6 hours per day, 7 days/week for 90 days. Livers were removed, homogenized and fractionated into crude nuclear, microsomal, plasma membrane, and cytosol fractions. Proteins were examined by SDS-PAGE analysis and immunoblotting for cytochrome P450 isozymes, apoptosis-related proteins, oncogenes, and other protein factors, and distinct changes in the expression of specific proteins identified. Proteomic data available from the same exposure groups allows a balanced interpretation of the changes in protein expression in the rat liver.

1801 MORPHOLOGICAL CELL TRANSFORMATION OF C3H10T½frac18½CL8 MOUSE EMBRYO CELLS BY THE K-REGION DIHYDRODIOL OF BENZO[*A*]PYRENE OCCURS IN COINCIDENCE WITH DNA DAMAGE BUT WITHOUT THE FORMATION OF DETECTABLE STABLE COVALENT DNA ADDUCTS.

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Benzo[*a*]pyrene (B[*a*]P) is the most thoroughly studied polycyclic aromatic hydrocarbon (PAH). Many mechanisms have been suggested to explain its carcinogenic activity, yet many questions still remain. K-region diols of PAHs are common metabolites and have been thought to be detoxification products. Here, we present evidence that the K-region diol of B[*a*]P, B[*a*]P-4, 5-diol, induced morphological cell transformation (MCT) in C3H10T½frac18½Cl8 mouse embryo fibroblasts (C3H10T½frac18½ cells). This diol also induced DNA damage in these cells but did not form stable covalent DNA adducts. B[*a*]P-4, 5-diol and B[*a*]P induced MCT in C3H10T½frac18½ cells as significant numbers of Type II and Type III foci were produced over a concentration range of 1.8-10.5 μM for B[*a*]P-4, 5-diol, and 2.1-8.1 μM for B[*a*]P. The dose-response curves for B[*a*]P-4, 5-diol and B[*a*]P were the same. Both B[*a*]P-4, 5-diol (8 and 10 μM) and B[*a*]P (1, 4, 8, 10 μM)induced DNA damage as determined by the comet assay after 1 hr of treatment without significant concurrent cytotoxicity. Furthermore, the distributions of comet tail contents were altered in the B[*a*]P-4, 5-diol and B[*a*]P treatment groups compared to the controls. DNA adduct patterns in C3H10T½frac18½ cells were examined after PAH treatment using ^{32}P-postlabeling techniques and TLC elution systems designed for polar adducts. While B[*a*]P treatment produced one major DNA adduct identified as B[*a*]P-DE-dGuo, no stable covalent DNA adducts were detected in the DNA of B[*a*]P-4, 5-diol-treated cells. In summary, this study provides evidence for the DNA damaging and MCT activities of the K-region diol of B[*a*]P in the absence of covalent stable DNA adducts. In concert with the reported MCT activities of K-region diols of other PAHs, these data suggest a new mechanism/pathway for the bioactivation of PAHs. This abstract does not reflect EPA policy

1802 A ROLE FOR HEAT SHOCK PROTEIN 90 (HSP90) IN INTRACELLULAR TRAFFICKING OF BENZO(A)PYRENE (BP) IN RAINBOW TROUT LIVER.

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Feeding rainbow trout 0.3 to 0.4 mg/kg/day of dieldrin for 9 to 12 weeks markedly alters the disposition of a subsequent dose of radiolabeled dieldrin and benzo(a)pyrene. After a single challenge dose of 10 umol/kg of [^{14}C]dieldrin or [^{14}C]BP the concentrations of their equivalents in bile increase 2 to 5-fold and 3 to 12-fold in visceral fat of dieldrin fed over control fish. This phenomenon can be partially explained by an increase in binding capacity and intracellular trafficking of [^{3}H]BP, rather than by microsomal metabolism. Size exclusion chromatography on Sephacryl S-300 HR of hepatic cytosol of control and dieldrin fed fish, separates a putative [^{3}H]BP binding protein aggregate that is greater than 300 kD. One component of this aggregate has been identified as HSP90 by electrospray ionozation mass spectroscopy. We propose that this aggregate traffics BP to sites of metabolism or secretion in hepatocytes. In low dose *in vivo* experiments, intracellular trafficking may be a rate limiting step for hepatic disposition of lipophilic xenobiotics.

1803 DMBA INDUCED APOPTOSIS IN MURINE preB CELLS IS DEPENDENT ON ACTIVATION OF CASPASE-8.

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Polycyclic aromatic hydrocarbons (PAHs) are known to be both carcinogenic and immunotoxic. We have demonstrated that DMBA, a prototypic PAH, causes apoptosis in a murine preB cell line (70Z/3) cocultured with a bone marrow stromal cell line (BMS2). We have also observed that *in vivo* treatment of C57Bl/6 mice with DMBA (50mg/kg) results in marked hypocellularity in the bone marrow. The aim of this study was to identify the specific apoptotic pathways activated by DMBA *in vitro* and *in vivo*. We observed caspase-8 cleavage in 70Z/3 cells following treatment with DMBA *in vitro*. Apoptosis was blocked by ZVAD-fmk and ZIETD-fmk, which are pan-caspase and caspase-8 specific inhibitors, respectively. Although a caspase-9 specific inhibitor (ZLEHD-fmk) failed to block cleavage of caspase-8, caspase-9 activation was blocked by the addition of the caspase-8 specific inhibitor (ZIETD-fmk). These results suggest that caspase-8 activation is upstream of caspase-9 activation in preB cells undergoing apoptosis as a result of exposure to stromal cell and DMBA metabolites. Typically, caspase-8 is activated in response to binding of death receptors by their cognate ligands. DMBA induced apoptosis was not blocked by the addition of a neutralizing mAb to FasL, indicating that the Fas/FasL system was not responsible for caspase-8 activation in our system. We have detected up-regulation of Protein Kinase R (PKR) a double stranded RNA dependent kinase that is capable of death receptor independent activation of caspase-8 in response to DMBA treatment. Caspase-8 activation has also been detected in the bone marrow of mice treated *in vivo* with DMBA, which may explain in part the hypocellularity that occurs in the bone marrow. By comparing our *in vitro* and *in vivo* systems we hope to better understand how caspase activation might lead to leukocyte apoptosis and the immunotoxic effects of DMBA.

1804 POLYCYCLIC AROMATIC HYDROCARBON MIXTURES: ASSESSMENT OF HUMAN HEALTH RISKS AND RESEARCH NEEDS.

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The Integrated Risk Information System (IRIS) Program has initiated a health assessment for chronic environmental exposure to polycyclic aromatic hydrocarbon (PAH) mixtures. Current health assessments and guidance that are used by EPA for estimating risks from exposure to PAH mixtures were developed in the early 1990s and include: 1) IRIS health assessments for 15 individual PAH; and 2) Provisional Guidance for Quantitative Risk Assessment of PAH (1993) which provides relative potency factors (RPF) for seven PAH identified as probable human carcinogens using BENZO(A)PYRENE (B[a]P) as a standard. Risk assessment of PAH mixtures has been hindered by a lack of information on the composition of mixtures, the toxicity of specific mixtures, the components that contribute most to toxicity, and the interactions and differences in mode of action of toxicity between components. This information is critical for the evaluation of risks for specific mixtures and the use of surrogates to estimate risk. More recent studies have emerged which, at least in part, address these issues; but the studies also point to data gaps and additional research needs. The status of the health assessment for PAH mixtures and a compilation of the identified research needs is presented which includes the following as exemplary data gaps: 1) oral and inhalation toxicity of various PAH mixtures and inhalation toxicity of B[a]P using standard chronic bioassays is lacking; 2) the use of B[a]P as a standard for assessing the toxicity of PAH mixtures is now questioned, but animal bioassays for potential surrogates (e.g., "supercarcinogens") and comparative *in vitro* studies of these PAH with other known components of PAH mixtures are minimal; and 3) although recent studies have provided more data regarding interactive effects among PAH, they are limited in scope with regards to PAH mixtures as a whole and the quantitative aspects have not been elucidated. (The opinions expressed are those of the author and should not be construed as EPA policy).

1805 POLYCHLORINATED BIPHENYLS-INDUCED APOPTOSIS OF MURINE SPLEEN CELLS IS ARYL HYDROCARBON RECEPTOR-INDEPENDENT BUT CASPASES-DEPENDENT.

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Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants, and many of the toxic effects including immunotoxicity are mediated by the activation of aryl hydrocarbon receptor (AhR). We previously reported that Aroclor 1254, one

of the most widely used PCB mixtures, increased DNA fragmentation in mouse spleen cells, assuming that apoptosis was correlated with the immunotoxicity of PCB (Yoo et al., 1997). In the present study we investigated the mechanism by which PCB induces apoptosis and the involvement of AhR in PCB-mediated apoptosis in mouse spleen cells. Aroclor 1254 induced DNA fragmentation without AhR activation, and the apoptosis was not affected by a-NF, a well-known antagonist of AhR. PCB congeners (PCB 47, 52, 128, and 153) that have little affinity for AhR induced DNA fragmentation, whereas congeners (PCB 77, 126, and 169) that have high affinity for AhR did not induce the fragmentation. Di-ortho form of PCB congener (PCB 153) and Aroclor 1254 induced DNA fragmentation in spleen cells of both AhR knockout mice (AhR-/-) and Ah low-responsive mice (DBA/2), whereas non-ortho form of PCB congener (PCB 126) did not induce the DNA fragmentation. In light of these findings, it is clear that AhR is not involved in the PCB-mediated apoptosis. PCB 153 significantly increased caspase-3 activity in both spleen cells and human leukemia (HL-60) cells. Z-VAD-fmk, general inhibitor of caspases, prevented the PCB-induced DNA fragmentation. Based on our findings, the most likely mechanism that can account for this biological effect involves the induction of caspase-dependent apoptotic cell death..

1806 EFFECTS ON PROLIFERATION, DIFFERENTIATION, AND GENE EXPRESSION OF BENZO(A)PYRENE, ARSENIC, AND CALCIUM ON NORMAL HUMAN EPIDERMAL KERATINOCYTES.

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We are interested in developing accurate risk assessment strategies for environmentally relevant chemical mixtures. To this end, normal human epidermal keratinocytes (NHEK) were chosen as an *in vitro* model for measuring the carcinogenic potential of petroleum-based hydrocarbons. Our initial studies examined the effects of BENZO(A)PYRENE (BaP), on proliferation and differentiation in NHEK; perturbations in these cellular processes have been strongly linked to carcinogenesis in keratinocytes. We have demonstrated that BaP inhibits terminal differentiation in NHEK, as measured by cornified envelope (CE) formation, by up to 5.8-fold in control cells and 1.8-fold in calcium (Ca) induced cells. In comparison, arsenic (As), a known inhibitor of this process, decreased CE formation by approximately 20-fold in control cells and 3.2-fold in Ca induced NHEK. We are interested in delineating the effects of BaP on both terminal growth arrest and subsequent expression of protein markers of keratinocyte differentiation. To this end, flow cytometric analysis is being used to measure cell-cycle distribution in BaP- and As-treated *versus* control populations and populations undergoing Ca-stimulated differentiation. BaP at 2μM has been shown to increase proliferation rates by 1.5-fold. In contrast, As at 1μM inhibited proliferation 1.6-fold; similar growth arrest is observed (1.4-fold) in NHEK treated with 2mM Ca. Our findings suggest that, although both BaP and As inhibit CE production in NHEK, different mechanisms may be involved. Current studies will attempt to identify, through microarray analysis, molecular markers involved in the observed chemical effects. Our aim is to correlate alterations in these molecular markers with the transformation process in NHEK, and to develop predictive capabilities for risk assessment of petroleum-based hydrocarbon mixtures. This research was supported by NIEHS Grant # RO1 ES09655 & Minority Supplement # RO1 ES09655-01S1.

1807 SUPPRESSION OF OVARIAN FUNCTION BY INHALED BENZO(A)PYRENE.

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This study was conducted to evaluate the effect of BaP on ovarian function. Adult female Fisher-344 rats were randomly assigned to a treatment and a control group per exposure regimen. Two treatment regimens utilized in this study, consisted of subacute exposure of rats *via* inhalation to 0.05 or 0.1mg m^3 of BaP:carbon black aerosol, four hours a day for 10 days. From the fourth day of exposure, animals in the control (unexposed) and treatment groups were synchronized with subcutaneous progesterone (P_4) injections (1 mg P_4/animal/day for 4 days). Folliculogenesis was induced in these animals with intra-peritoneal (IP) injection of 15 IU of equine gonadotropin (EG) about 24 hours after the last P_4 injection [day 7 of BaP treatment]. Ovulatory response was subsequently induced at 48 hours post EG by an IP injection of 15 IU of human chorionic gonadotropins [hCG; day 9 of BaP treatment]. Twenty four hours post cessation of exposure of animals to BaP, all animals in the study were sacrificed, ovaries excised and weighed and number of ovulation points per animal determined by counting the total number of corpora lutea on both ovaries. All rats responded identically to the synchronization and folliculogenesis/ovulation induction protocol. Both regimens of BaP supressed ovulation rates ($P<0.05$) compared with controls (0.05 mg BaP/m^3, 11.0 ± 2.4 vs 24.4 ± 1.6; 0.1mg BaP/m^3, 18.7 ± 1.5 vs control, 29.0 ± 1.3) The percentage suppression of ovulation rate by BaP was exposure concentration-dependent ($P<0.05$). However, regardless of BaP-exposure concentration, ovarian weights were unaffected. These data suggest that (a) BaP perturbs the ability of fixed exogenous gonadotropins to adequately initiate folliculogenesis and ovulation and (b) BaP probably acts to reduce ovulatory response to gonadotropins *via* a reduction in follicular steroidogenesis (Supported by MHPF/ATSDR grant U50/ATU398948).

1808 ROLE OF BENZO(A)PYRENE (BAP) METABOLISM IN LUTEOTROPIC ACTIVITY AND FETAL SURVIVAL OF TIMED-PREGNANT RATS EXPOSED TO BAP: CARBON BLACK AEROSOL.

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This study was undertaken to assess the effect of BaP metabolites on luteal maintenance and fetal survival in timed-pregnant Fisher 344 rats. Treatment consisted of subacute nose-only inhalation exposure of rats to 25, 75, and 100 μg/m^3 of BaP: carbon black aerosol, for 4 hours a day for 10 days, beginning on day 10 of gestation. Control animals were either sham exposed to carbon black (CB) or unexposed (UNC). Blood samples were collected on days 15, 17, and 20 of gestation *via* ocular puncture for plasma. Number of pups per litter was determined postpartum. Luteotropic factor in plasma samples was determined by measuring prolactin (PRL) levels by RIA. Metabolite levels in plasma and tissue samples from pregnant rats, and pups (postnatal day 1) were measured and identified by reverse phase HPLC coupled with fluorescence detection. Fetal survival declined ($P<0.05$) in a concentration-dependent manner as a result of exposure of rats to BaP (25μg/m^3 73%; 75 μg/m^3 38%; 100 μg/m^3 31%) compared with CB (77%) and UNC (86%). Plasma PRL concentrations in BaP treated rats showed a concentration-dependent decline relative to controls. The metabolite levels in plasma, uterine tissues, and pups were also exposure concentration-dependent. These results indicate a significant correlation ($P<0.05$) between plasma, tissue metabolite levels of mothers and fetal survival, and also between plasma metabolite and PRL levels. Further, these results strongly suggest that the exposure concentration-dependent disposition of inhaled BaP metabolites, especially the trans-7, 8-dihydrodiol contributed to a suppression in luteotropic activity and reduced embryonic survival (Supported by MHPF/ATSDR grant U50/ATU398948, NIH grant G12RRO3032, SCOR project 2SO6GMO8037-28, and RCMI project RR/AI 0303271).

1809 DIFFERENTIAL EFFECTS OF POLYCHLORINATED BIPHENYLS (PCBs) ON EXPRESSION OF HEPATIC ANION TRANSPORTERS Oatp2 AND Oatp3 AND MULTIDRUG RESISTANCE PROTEIN 2 (Mrp2) IN RATS.

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Transfer of thyroxine (T_4) across plasma membranes is accomplished, in part, *via* transport proteins. Two proteins possibly involved in this process are organic anion transporting polypeptides Oatp2 and Oatp3. Additionally, Mrp2, a member of the superfamily of ATP binding cassette transporters, is implicated in the excretion of T_4-glucuronide (T_4-G) into bile. The purpose of this study was to determine whether PCBs, which markedly reduce serum T_4, might do so by enhancing the hepatic uptake of T_4, and subsequent excretion into bile, *via* increases in the expression of these proteins. Male rats were administered Aroclor 1254 or Aroclor 1242 for 7 consecutive days and 24 hrs after the last dose, [^{125}I]-T_4 was administered iv. At 1, 3, 5, 10, 15 and 30 min post-injection, blood and liver were sampled. The [^{125}I]-T_4 present in serum and liver was quantified by gamma spectrometry, and transporter protein levels in liver were determined by western-blot analysis. Within one min post-injection, Aroclor 1254 produced a 56% decrease in serum [^{125}I]-T_4 and Aroclor 1242 a 30% reduction within 3 min, as compared to controls. At the one min time point, Aroclor 1254 and Aroclor 1242 produced a 105% and a 54% increase, respectively, in the uptake of [^{125}I]-T_4 into liver, as compared to the control value. Oatp2 protein levels were suppressed 87% by Aroclor 1254, compared to the control, but Aroclor 1242 had no significant effect on Oatp2. No significant changes in Oatp3 protein levels were observed. In contrast, Aroclor 1254 and Aroclor 1242 produced significant increases in Mrp2 protein levels of 108% and 92%, respectively, relative to control. These data suggest that the increase in hepatic uptake of T_4 in rats exposed to PCBs cannot be explained by upregulation of Oatp2 and Oatp3. However, upregulation of Mrp2 may contribute to increased biliary excretion of T_4-G. (Supported by NIH grants ES-07148, ES-05022 and ES-08156)

1810 MODULATION OF ACUTE NEUROBEHAVIORAL TOXICITY OF BENZO(A)PYRENE BY METABOLISM, ANTIOXIDANT ENZYMES, AND NEUROCHEMICAL PARAMETERS IN F-344 RATS.

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The objective of this study is to understand the effects of Benzo(a)pyrene (BaP) on perturbation of nervous system by correlating behavioral changes with metabolism, antioxidant enzyme activities, and neurochemical parameters in selected brain regions. The neurobehavioral effect of single oral doses (12.5-200 mg/kg) of BaP on motor activity and the functional observational battery (FOB) were studied in male F-344 rats. Immediately after dosing, animals were returned to Digiscan activity cages and monitored for locomotor activities, beginning at 1800 hrs at 2 hrs and for a total of 12 hrs for five consecutive days. The FOB was conducted at 2, 6, 12, 24, 48, 72 and 96 hrs post BaP treatment. Benzo(a)pyrene parent compound and metabolites in plasma and brain tissues were measured by reverse phase HPLC. The effects of BaP on dopamine levels in the striatum were examined at 24 and 48 hrs post treatment by HPLC. Concentrations of antioxidant enzymes [catalase, superoxide dismutase (SOD), and glutathione peroxidase] were determined at 6 and 96 hrs in both the striatum and hippocampus of BaP exposed rats. Locomotor activity was suppressed on day 2 with recovery to control levels by day 3 in treated rats. Behavioral effects reached a maximum at 6 hrs in all FOB test measures but were reversible after 72 hrs in all dose groups. Bioavailability data suggest a strong link between BaP metabolism and the onset and duration of behavioral effects. A dose-dependent decrease in striatal dopamine content was noticed in treated rats. Benzo(a)pyrene produced a 30-50% and 12-25% upregulation of SOD at 6 and 96 hrs post treatment respectively. These findings suggest BaP cause acute neurobehavioral toxicity and modulation of striatal dopamine levels. Furthermore, the alteration of free radical status may contribute to BaP- caused neurotoxicity (Supported by MHPF/ATSDR U50ATU398948, NHLBI T32-HL07809, US Army DAMD-17-99-1-9550 and NIH 2506-GM-08037).

1811 REPRODUCTIVE PERFORMANCE OF TWO GENERATIONS OF RATS EXPOSED TO LOW LEVELS OF TECHNICAL CHLORDANE.

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Technical chlordane is a persistent organochlorine mixture of four main isomers (heptachlor, cis-chlordane, trans-chlordane, and trans-nonachlor). The effects of chlordane at environmental levels on reproductive performance of humans and other mammals are relatively unknown. Sprague-Dawley rats (F0, F1 & F2) were used in a two-generation study to evaluate technical chlordane effects on fertility index, pregnancy outcome, indices of puberty, reproductive organs weight and morphology, vaginal cytology and testicular parameters. Chlordane doses were 0.0, 0.125, 0.250 & 1.0 mg/kg body weight/day. Results of the first of two replicates indicated that, chlordane delayed ($P<0.05$) the ontogeny of vaginal opening in F1 females, but time to first estrus was unaffected; in contrast, F2 generation manifested no effect in time of vaginal opening, but time to first estrus was delayed at 1.0 mg/kg level. Estrus cyclicity was not affected. Time of preputial separation was advanced in F1 males at 0.125 mg/kg/day. Relative to the control, mating index was decreased in F1 chlordane treated groups, but not in F0. There was no treatment effect on total litter weight, litter size and sex ratio in both F1 and F2 generations. At necropsy, chlordane increased uterine weight in F0 dams at 0.125 mg/kg level and reduced testicular weight of F2 males at 0.250 mg/kg level. Measurement of the semeniferous tubules revealed an increase in its diameter and epithelial height of F1 & F2 chlordane treated groups. Sperm cells motility, velocity, morphology and concentration as well as plasma testosterone levels were unaffected. These results indicated that low levels of technical chlordane disrupted the normal morphology and physiology of the reproductive organs. (Supported by: MHPF/ATSDR-#U50/ATU3922660008)

1812 REPRODUCTIVE TOXICITY ASSESSMENT OF CHRONIC ORAL ADMINISTRATION OF FLUTAMIDE IN MALE RATS.

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Flutamide [2-methyl-*N*-(4-nitro-3-[trifluoromethyl]phenyl)propanamide] is a non-steroidal antiandrogen used as a therapeutic agent for prostate cancer. We have recently demonstrated that chronic dietary administration of flutamide also inhibits prostate carcinogenesis in a rat model. The present study was performed to determine the effects of a chemopreventive flutamide regimen on male reproductive parameters. Groups of 10 male Wistar-Unilever rats (9 - 10 weeks of age) were fed a basal chow diet or basal diet supplemented with flutamide (25 or 100 mg/kg diet) for $\geq$ 12 months. In comparison to dietary controls, rats exposed to flutamide demonstrated significant reductions in mean testes and epididymis weights. A non-monotonic dose-response was seen: testicular atrophy was present in 10/10 rats in the low dose flutamide group, but only in 4/10 rats in the high dose group. Similarly, although both doses of flutamide reduced spermatid number and daily sperm production, the low dose had quantitatively greater effects. Epididymal smears obtained from flutamide-treated rats demonstrated relatively few sperm; greater reductions in epididymal sperm count were seen at the low flutamide dose than at the high dose. Percent abnormal sperm (kinky tail, absence of head) was increased only in samples obtained from rats receiving the low dose of flutamide; percent abnormal sperm in the high dose group was comparable to that in dietary controls. Testicular histopathology in flutamide-treated rats demonstrated small seminiferous tubules populated primarily by spermatogonia and spermatocytes; relatively few spermatids were present. These data suggest that flutamide doses that confer significant protection against prostate cancer induction are likely to have significant adverse effects on male fertility. Decisions regarding the possible use of flutamide in prostate cancer prevention must include considerations of the impact of this agent on sexual potency.

1813 TRANSGENIC RATS AS A MODEL FOR THE INVESTIGATION OF ESTROGENIC EFFECTS.

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Endocrine disruptors are environmental chemical which may disrupt reproductive development.The ability xenooestrogens to bind to the estrogen receptor (ER) has been used as a definition of estrogenicity.Data suggest that endocrine disruptive effects are due to the interaction of the xenoestrogen with ER that regulate gene expression.Estrogenic chemicals typically bind the ER, the ER-ligand complex interacts with estrogen response elements (ERE), and transcription factors are recruited to the ER-ligand/ERE complex, resulting in enhanced gene expression.Receptor/ligand binding causes changes in gene expression leading to altered function of endocrine-regulated physiological systems.The inital screening methods used to detect estrogenic/anti-estrogenic activity are combinations of ER binding activity and transcriptional cellular assay systems *in vitro*.The interpretation of these data in the context of the *in vivo* situation can be difficult.The purpose of our study was to establish a transgenic animal approach, which allows to investigate transcriptional changes with functional alterations in the same animal.For this purpose, we investigated ERE-containing promotor-reporter usage *in vitro*.Reporter assays revealed dose-dependent response curves after treatment of the MCF-7 cells with a series of 17beta–estradiol and Bisphenol A concentrations. After *in vitro* testings, we established different transgenic rat strains on a Sprague-Dawley background using a pS2 promoter-construct.Mating of the founder generation with wild type Sprague-Dawley rats revealed transgenic positive rats.RT-PCR analysis of mRNA revealed reporter-gene expression in different organs (e.g. uterus) of untreated rats from different transgenic strains postnataly.Here we established a transgenic ERE-reporter rat as a model for testing estrogenicity of endocrine disruptors *in vivo*.Using this transgenic rat strain we have the posibillity to study the effects estrogens and xenooestrogens on endocrine-regulated physiological systems.

1814 IDENTIFICATION OF A CONSUMER PRODUCT ADDITIVE THAT INHIBITS OVIDUCTAL FUNCTIONING IN PICOMOLAR DOSES.

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Our past studies have shown that chemicals in cigarette smoke inhibit oviductal functioning *in vivo* and *in vitro* (Magers, Reprod Toxicol 1995, 9:513; Knoll, Reprod Toxicol 1998, 12:57). The purposes of this study were to identify the individual toxicants in cigarette smoke solutions made from 2R1 research brand cigarettes (University of Kentucky) that inhibit oocyte pickup rate, ciliary beat frequency, and infundibular smooth muscle contraction, and to determine their effective doses. Solid phase extraction and gas chromatography-mass spectrometry (GC-MS) were used to identify individual chemicals in the mainstream and sidestream cigarette smoke solutions. Pyridine derivatives were the most abundant class of compounds identified by GC-MS. Commercially available pyridines were purchased, assayed for purity, and tested in dose-response studies on the oviduct using an *in vitro* bioassay. The no observable adverse effect level (NOAEL) and lowest observable adverse effect level (LOAEL) were determined for each pyridine compound using the oocyte pickup rate, ciliary beat frequency, and infundibular mus-

cle contraction assays. 2-ethylpyridine, 2-methylpyridine, and 3-ethylpyridine were inhibitory at picomolar concentrations in all assays. In the 2R1 cigarettes, 3-ethylpyridine was found in smoke, but not in unburned cigarettes. 3-ethylpyridine is one of 599 chemicals added to commercial cigarettes to enhance flavor(http:\www.rjrt.com/TI/Pages/Ticig_ingred_Summary.asp). Two commercial cigarette brands were analyzed and significant amounts of 3-ethylpyridine were found in the unburned tobacco as well as in the mainstream and sidestream smoke. 3-ethylpyridine is also used as an additive in candy, ice cream, meat, and baked goods and is on the Flavor and Extract Manufacturers' Association's GRAS "generally regarded as safe" list, and the FDA's EAFUS (everything added to food in the United States) list. This work shows that very low doses of 3-ethylpyridine significantly inhibit proper oviductal functioning.

1815 LACK OF REPRODUCTIVE TOXICITY IN A 2-GENERATION STUDY OF DI(2-ETHYLHEXYL)TEREPHTHALATE (DEHT, DOTP) IN RATS.

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A two-generation reproductive toxicity study of DEHT was conducted in which groups of 30 male and 30 female Sprague-Dawley rats were exposed to 0, 0.3, 0.6, or 1.0% DEHT in the diet. The F0 animals received the diets for at least 70 days prior to mating and until termination of the generation; the F1 generation received diets beginning on PND 22. All females were allowed to deliver and rear their pups to weaning (lactation day 21). Mean maternal body weights and body weight gains were reduced for F0 and F1 females in the 1.0% group throughout gestation. Mean maternal body weights were also reduced for the F0 and F1 females in this group throughout lactation. Overt toxicity and mortality were seen in the 1.0% F0 and F1 groups. Increases in absolute (F0 females) and mean relative (to body weight) liver weights (females in both generations) were observed for the 0.6 and 1.0% groups. No test article-related microscopic findings were observed in any of the tissues examined for either sex in either generation. Reproductive parameters (fertility, mating, days between pairing and coitus, gestation, parturition, and estrous cycling) were unaffected by treatment at any dose level during the F0 and F1 generations. Mean litter sizes, numbers of pups born, percentages of males per litter at birth and postnatal survival were unaffected by parental treatment at all concentrations. F1 and F2 offspring weights in the 0.6 and 1.0% groups were reduced throughout the pre-weaning period for the F1 generation and beginning on PND 4 and 7 for the F2 generation in the 0.6 and 1.0% groups, respectively. The NOAEL for reproductive toxicity was considered to be 1.0%. The NOAELs for parental toxicity and neonatal toxicity were considered to be 0.3%. These data complement the information from Gray et al. (2000) that indicate that DEHT does not produce antiandrogenic effects.

1816 EFFECTS OF PRENATAL TESTOSTERONE PROPIONATE AND VINCLOZOLIN ON PERINATAL AND INFANTILE DEVELOPMENT OF MALE AND FEMALE RATS.

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Prenatal exposure to both androgenic and antiandrogenic chemicals can adversely effect pregnancy and growth of offspring, but have opposite effects on sexual development. We conducted a study to determine whether an antiandrogenic chemical, vinclozolin (V), and an androgenic chemical, testosterone propionate (TP), in combination or alone, have similar or opposing effects on perinatal health and reproductive development. Sprague-Dawley rat dams were dosed on gestational day (GD) 14 (GD 1 = day of plug) to GD 19 with either corn oil (vehicle, orally), V (200 mg/kg, orally), TP (1 mg/rat, sc injection), or V+TP. Weight gain in dams through the dosing period was reduced by V (35.4 g, $p < 0.05$ *vs.* 53.1 g in controls) and by TP (28.1 g; $p < 0.005$) but was further reduced by V+TP (21.9 g, $p < 0.0005$). Live litter size on postnatal day (PND) 2 was reduced by V+T only (5.6, $p < 0.001$ vs 13.8 in controls), although mean number of uterine implantation sites was not affected. Anogenital distance (AGD) in females on PND 2 was increased from control values (1.73mm) by TP (2.51mm; $p < 0.0001$) and restored by co-administration of V (1.62). AGD in males was reduced from control values (4.04 mm) by V (2.18; $p < 0.0001$), but this reduction was not antagonized by co-administration of TP (2.07mm). Number of areolas on PND 14 in females was reduced from control values (12.05) by TP (3.69; $p < 0.0001$) and fully restored by V. Areolas were induced by V in males and co-administration of TP partially attenuated the prominence of the areolas (score = 23, scored by prominence on a scale of 1 - 36 per pup; $p < 0.0001$) compared to those in the V group (score = 30). We conclude that V and TP are additive in maternal and pup health, V attenuates the androgenic effects of TP on AGD and areolas in the female rat offspring, but the dose of TP used here does not fully attenuate the antiandrogenic effects of V on AGD and areolas in male rat offspring. This abstract does not necessarily reflect USEPA policy.

1817 DIBROMOACETIC ACID (DBA) ALTERATIONS IN OVARIAN STEROIDOGENESIS: SITE(S) OF IMPACT ON PROGESTERONE (P4) SECRETION.

J. M. Goldman and A. S. Murr. *Endocrinol Br, Repro Toxicology Div, USEPA, Research Triangle Park, NC.* Sponsor: A. Cummings.

Chlorination of public drinking water is among the most successful of treatments to combat disease. However, various generated byproducts have been linked to certain types of cancer & reproductive alterations in test animals. Haloacetic acids are a major class of these byproducts, & at least 2, DBA & dichloroacetic acid, are reported to alter sperm production and gonadal hormone activity in male rats. In females, DBA (90 & 270 mg/kg, po) disrupted cyclicity, an effect possibly related to a fall in P4 secretion (seen *in vitro*). To explore this further, 26d Sprague-Dawley rats given 15IU of pregnant mare serum gonadotropin were killed 48h later. Preovulatory ovarian follicles from 12-13 rats (per incubation) were removed & pooled. They were paired in oxygenated Medium 199 (M199, 37°C) with added BSA, HEPES & gentamicin sulfate within Teflon® vials under 1 of the following conditions: M199, DBA (50 µg/ml), hCG (100 IU/ml), or DBA+hCG (all pH-adjusted to 7.4). The DBA concentration reflected a mean 5h circulating value after a dose (270 mg/kg, po) that reliably disrupted cyclicity. Samples were taken at 4, 8, 12 & 24h. As before, 50 µg/ml lowered non-stimulated (NS) & hCG-stimulated (STIM) P4 release, an effect mirrored by the follcular P4 content at 24h. Estradiol release was unchanged. No effects on NS or STIM P4 release were seen following inclusion of pregnenolone (Pregn, 1 µg/ml), indicating tissue viability & Pregn conversion to P4 were not affected. Addition of 22(R)-hydroxycholesterol (22R-HC, 20µM) (circumventing intramitochondrial cholesterol transport by steroidogenic acute regulatory protein [StAR]) showed no difference in NS P4 release between DBA & non-DBA controls, implicating an effect on StAR in P4 suppression. Whether the alteration in cyclicity is linked to a decrease in P4 is uncertain. However, the P4 antagonist RU486 will cause such disruptions in cyclicity, suggesting that an effect on P4 secretion may at least partially contribute to the observed alteration in cyclicity. (This abstract does not reflect EPA policy.)

1818 *IN UTERO* EXPOSURE TO ATRAZINE INDUCES DELAYED PUBERTY OF LONG EVANS RATS: DAM-MEDIATED EFFECTS IN FEMALES.

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Exposure of Wistar dams to the common herbicide atrazine (ATR) on postnatal days (PND)1-4, will delay vaginal opening (VO) in the offspring, without reduced body weight (BW). We evaluated the effect of gestational ATR on puberty induction and compared whether effects were strictly dam-mediated or a direct effect on the pups. Time pregnant Long Evans rats (N=20/group) were gavaged gestational days (GD) 15-19 with 100 mg ATR/kg or methylcellulose (controls, C). BW of the ATR dams on GD19 was significantly reduced (346±7g), when compared to control dams (396±7g). On PND1, half litters were cross-fostered, creating 4 treatment groups; C-C, C-ATR, ATR-C, and ATR-ATR (milk-birth source, respectively). BW was compared on PND4, PND22, day of VO or preputial separation (PPS), and PND60 (female) or PND120 (male). A significant delay in VO was observed in females fostered to ATR-treated dams (Mean±SE, N>30 pups each group); C-C 33.7±0.4d, C-ATR 33.3±0.4d, ATR-C 35.4±0.6d, and ATR-ATR 37.1±0.5d. BW was significantly reduced at PND4 (N>35 pups in each group) in ATR-ATR animals *vs.* C-C (9%) or C-ATR (9%), but was not different between those dose groups at weaning or PND60. Although VO was delayed by ATR, the BW at VO of ATR-ATR (146.8±4.4) and ATR-C offspring (140.0±4.4) was greater than C-C (130.6±2.8). Male pups exposed to ATR *in utero* (N>18/dose group) displayed delayed PPS whether they suckled from an ATR (43.8±0.5d, ATR-ATR) or control dam (42.5±0.4, ATR-C). However, PPS was similar between C-ATR(41.5±0.4) and C-C (41.3±0.3). The ATR-ATR and ATR-C males were reduced in BW 9% and 6%, respectively, from that of C-C at PPS. Our data suggests that the delay in VO in offspring exposed to ATR *in utero* is mediated *via* the dam, and does not appear to be caused by decreased BW. Whether these effects are the result of a hormonal or lactational alteration requires further investigation. *(This abstract has been reviewed in accordance with USEPA policy and approved for publication)*

1819 ABBREVIATED ONE-GENERATION STUDY OF DIETARY BISPHENOL A (BPA) IN CD-1® MICE.

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This study duplicated the species/strain, route, and top 2 dietary BPA concentrations (5000 and 10, 000 ppm) employed in a previous RACB study (Reel et al., 1985), but with shorter duration, one breeding, and enhanced evaluation of parental systemic/reproductive toxicity to provide the context for previously reported F1 offspring effects at these doses. F0 mice (20/sex/group) were exposed ad lib to BPA for 2-wk prebreed; 1-wk mating (F0 males necropsied after mating; 3-wk total exposure); and ca. 3-wk gestation. F0 dams and F1 litters were necropsied at parturition (pnd 0). Parental systemic toxicity was present at both BPA doses: increased liver and kidney weights (WT) in both sexes; for F0 dams: reduced body weights (BW), weight gain, and feed efficiency during pregnancy; prolonged gestation (no effects on precoital interval or ovarian or uterine WT); histopathology in liver (dose-related hepatocellular hypertrophy) and kidneys (dose-related tubular epithelial degeneration, necrosis and regeneration); elevated serum BUN at 10, 000 ppm; and reduced serum Na, K, and Cl at 5000 ppm (consistent with renal toxicity). F1 offspring toxicity was observed only at 10, 000 ppm: slightly reduced total and live pups/litter, with no effects on pre-/postimplantation loss or pup BW. This study resulted in comparable daily BPA intakes (for both sexes and doses) and confirmed litter size effects at 10, 000 ppm by Reel. It also demonstrated parental systemic toxicity at both doses. Our data support the interpretation of litter size effects at 10, 000 ppm as caused by the compromised status of dams (after 5-6 wks total exposure), while the lesser systemic toxicity at 5000 ppm did not cause litter size effects. F1 litter size effects at 5000 ppm were observed by Reel only for 4th-5th litters after much longer exposure duration (>90 days).

1820 ALTERATIONS IN THE RAT ESTROUS CYCLE BY DIBROMOACETIC ACID (DBA): RELATIONSHIP TO A SUPPRESSION IN ESTRADIOL (E2) METABOLISM?

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Of the large variety of chemicals formed by chlorination of public drinking water, a number are suspected to cause reproductive alterations in humans and test animals. Members of one class, the haloacetic acids (of which DBA is a member) have been reported to alter rat sperm production & gonadal hormonal activity at elevated doses. In females, DBA was found to induce a persistent alteration in estrous cyclicity, something found in the present study using 14d exposures (0-270 mg/kg, po). Body wts were unaffected. While a fall in progesterone (seen *in vitro*) could impact cyclicity, it may be that E2 levels are also altered. *in vitro* data did not show shifts in ovarian E2 release, but an impact on E2 metabolism could affect blood levels. Consequently, cycling female rats were treated (po) daily for 2 wks with DBA (0, 30, 90, 270 mg/kg in water, pH adj. to 6.8). After 8d (during which cycles continued), females were ovariectomized & 3d thereafter implanted with estradiol capsules (OVX-E2). Daily tail nick blood was then taken for 3d. Non-E2 capsules were also implanted & blood taken (0 & 270 mg/kg DBA). Additional OVX-E2 rats (270 mg/kg) received phenobarbital (PB, 0.1% in drinking H_2O) for 4d before sampling to stimulate E2 metabolism. After capsule implantation, an initial serum E2 rise then fell over the next 2d. By 72h, DBA caused a dose-related increase in E2 that at the highest dose was approx. 2 1/2-fold above controls. PB reduced DBA-elevated E2 levels. Data suggest that alterations in cyclicity seen following DBA exposures could be at least partially due to a suppression in hepatic E2 metabolism. The rise in E2 is reversible & speaks against suicide inhibition of the relevant P450 activity. The data indicate that DBA given at these doses over 2 wks acts as an endocrine disrupting chemical. While it is conceivable that lower doses over more extended periods could have similar effects on reproductive functioning, such studies remain to be conducted. (This abstract does not reflect EPA policy.)

1821 EFFECTS OF METHOXYCHLOR (M) OR ITS ACTIVE METABOLITE, 2, 2-BIS (P-HYDROXYPHENYL)-1, 1, 1-TRICHLOROETHANE (HPTE), ON TESTOSTERONE (T) FORMATION BY CULTURED ADULT RAT LEYDIG CELLS (LC).

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M is a pesticide developed as a replacement for dichlorodiphenyltrichloroethane (DDT). Its active metabolite is reported to be HPTE. Both M and HPTE have been shown to exhibit weak estrogenic or antiandrogenic activities in various *in vivo* or *in vitro* testing protocols. In the present studies, we examined the direct effects of M or HPTE on T biosynthesis by cultured LC from young adult rats. Increasing concentrations of M or HPTE (1-1000 nM) caused a progressive decline in both basal and 10 mIU/ml human chorionic gonadotropin (hCG)- or 1 mM 8-Br-cAMP-stimulated T following exposure for 4 or 24 h, beginning at 100 or 500 nM, although the declines with HPTE generally were greater. To localize the site(s) of action of HPTE, LC were exposed to HPTE (1-1000 nM) alone for 24 h, then fresh media containing steroid precursors of T were added to follow the ability of exposed cells to convert these substrates to T over 4 h. The conversion of 0.01 mM pregnenolone, progesterone, or androstenedione to T was unaffected by prior exposure to HPTE; however, there was a progressive decline in the conversion of 0.01 mM 22(R)-hydroxycholesterol to T, suggesting that among the enzymes involved in the conversion of cholesterol to T, P450 cholesterol side-cleavage activity is inhibited by HPTE. The concomitant inclusion of the pure estrogen antagonist, ICI 182, 780, did not alter the inhibitive effects of HPTE, suggesting that the actions of HPTE are not mediated by binding to estrogen receptor alpha or beta. Also of interest, the addition of the native estrogen, 17beta-estradiol, or the antiandrogens, cyproterone acetate (1-1000 nM) or hydroxyflutamide (1-1000 nM), had no effect on basal or hCG-stimulated T following exposure for 24 h, suggesting that the actions of HPTE are not due to its estrogenic or antiandrogenic properties.

1822 EXPRESSION OF BIOTRANSFORMATION ENZYMES IN OVARIAN INTERSTITIAL CELLS: EFFECT OF DOSING WITH 4-VINYLCYCLOHEXENE AND ITS DIEPOXIDE METABOLITE IN MICE.

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Our laboratory is investigating the role of ovarian metabolism in 4-vinylcyclohexene-induced ovotoxicity in mice. This study was designed to evaluate the effect of repeated dosing with the ovotoxicants 4-vinylcyclohexene (VCH) and its diepoxide metabolite (VCD) on expression of several biotransformation enzymes in the ovarian interstitium, whose function is poorly understood. Female B6C3F$_1$ mice (d28) were dosed daily (15d) with vehicle (Con), VCH (800 mg/kg) or VCD (80 mg/kg). Ovaries were removed and fixed for immunohistochemistry, or isolated into interstitial cells. We evaluated the effects of VCH/VCD dosing on mRNA expression and protein distribution of cyp 2e1, cyp 2a4/5, and microsomal epoxide hydrolase (mEH) using realtime PCR or confocal microscopy, respectively. Luteinizing hormone receptor was used as a marker for confocal visualization of interstitial cells. mRNA encoding *cyp* 2e1, *cyp* 2a4/5, and *mEH* was present in interstitial cells from control mice. However, after repeated dosing, expression was significantly decreased only for *mEH* (60±37% of Con, VCH, $p<0.05$), compared to control. Intense immunostaining for cyp 2e1, cyp 2a4/5, and mEH proteins was seen in interstitial cells from control mice. VCH dosing decreased staining intensity for cyp 2e1 (19±2.4% of Con VCH, $p<0.05$), while increasing staining intensity (39±5.1% above Con VCH, $p<0.05$) for cyp 2a4/5, compared to control. Although not significant, there was a downward trend in staining intensity for mEH, (20±10.3% of Con VCH, 32±19.3% of Con VCD) compared to control. Taken together, mRNA and protein for several biotransformation enzymes were investigated in ovarian interstitial cells. Their expression was affected by repeated dosing with VCH/VCD. Thus, these cells may play an important role in the ovarian metabolism of xenobiotics. (ES08979, ES06694, ES07019)

1823 EFFECT OF BLOCKADE OF GLUTATHIONE (GSH) SYNTHESIS ON OVARIAN FOLLICULAR ATRESIA.

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The mammalian ovary contains a finite complement of oocytes, most of which are destined to undergo the apoptotic process of degeneration termed follicular atresia. Our previous work has shown that healthy, growing follicles, but not atretic follicles, express high levels of the modulatory subunit of glutamate cysteine ligase (GCL), the rate limiting enzyme in the synthesis of the antioxidant tripeptide GSH (Luderer et al, 2000, Toxicol Sciences:54:257). Depletion of cellular GSH causes apoptosis in many cell types; however, the effect of GSH depletion on ovarian follicular atresia has not been investigated. To test the hypothesis that GSH depletion causes increased follicular atresia, cycling adult rats were treated with i.p. injections of 5mmol/kg buthionine sulfoximine (BSO), a specific inhibitor of GCL, in saline or with saline alone at 0700 and 1900h on proestrus (n=2, saline; n=3, BSO) or estrus (n=3 saline; n=4, BSO), and sacrificed at 0700h the next day. One ovary was homogenized for total GSH assay using the Tietze method. The other ovary was cryosectioned and subjected to terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) using the Cell Death Detection Kit (Roche

Molecular Biochemicals, Mannheim, Germany) to detect apoptotic cells and counterstained with hematoxylin. Follicles were also scored for atresia using histological criteria. Logistic regression analysis was used to examine the effect of BSO treatment on atresia while adjusting for estrous cycle stage and follicle type. Ovarian GSH concentrations in BSO-treated animals were 50% lower than in saline treated animals. BSO treatment was associated with a statistically significant increase in follicular atresia as determined by histological criteria and a non-significant increase in follicles containing apoptotic cells by TUNEL. Estrous cycle stage and follicle type were also significantly associated with atresia, with antral follicles from metestrous ovaries most likely to be atretic. These results suggest that ovarian GSH plays a role in preventing follicular atresia.

1824 COMPARISON OF THE EFFECTS OF MT-1E AND MT-3 OVEREXPRESSION ON GROWTH INHIBITION AND CHEMOTHERAPEUTIC DRUG RESISTANCE IN THE PROSTATE CANCER CELL LINE, PC-3.

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The third isoform of metallothionein (MT-3) has been shown to be overexpressed in prostate cancers and PIN lesions. The expression of MT-3 is highly variable but appears to correlate to the type and grade of the tumor. The expression of MT-3 in prostate cancer was modeled by stably transfecting the commonly utilized prostate tumor cell line, PC-3 with MT-3. Previously it was shown that this caused an inhibition of cell growth and an increased resistance to the chemotherapeutic drugs, vinblastin, etoposide, mitoxantrone, cisplatin, and paclitaxel as assess by the colony formation assay. The PC-3 cell line was also stably transfected with another commonly expressed MT isoform in the prostate that is not expressed in this cell line, MT-1E, to see if the above effects are specific to MT-3 or a general feature of all metallothioneins. Five MT-1E expressing clones were picked for further analysis and the level of the MT1/2 protein was found to be increased from 0.98±0.1 to 5.81±0.14 ng MT/μg protein as assessed by immuno-dot blot analysis. The MT-1E expressing clones had similar resistance profiles to the above chemotherapeutic drugs as the MT-3 expressing clones, but did not show any change in the growth rate of the cells. The mean doubling time the MT-1E expressing clones was 39.2±3.8 hr compared to 34.6±1.9 hr for the blank vector controls whereas the MT-3 transfected clones had a mean doubling time of 63.4±2.2 hr. These data indicate that the effect of MT-3 on cell growth is a unique feature of the MT-3 isoform probably facilitated by the unique sequence in the beta domain of the protein whereas the effect on the resistance to chemotherapeutic drugs is a common characteristic of the MT isoform family.

1825 TIME-COURSE ALTERATIONS IN CDK FAMILY GENES IN GERM CELL APOPTOSIS INDUCED BY 2-BROMOPROPANE (2-BP) IN RAT.

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Epidemiological surveys and animal experimental studies suggested that exposure to 2-BP could develop reproductive and hematopoietic disorders both in human and experimental animals. In our previous studies, we confirmed that 2-BP exposure results in apoptotic death of testicular germ cells and this process involves the Bcl-2 family genes and the Fas signaling system. In this study, we further explored the roles of cyclin dependent kinase (CDK) family genes in the development of male germ cells degeneration. Sixty-four rats were divided into eight groups, and percutaneously injected with a dose of 1350 mg/kg of 2-BP for 1 to 5 days. The rats were time-serially euthanized at 6 h after1, 2, 3, 5 doses, and 2 or 7 days after final treatments. Histological examination, TUNEL staining of DNA fragments, and Western blotting analysis of CDK family proteins (Cyclin E, CDK 2, CDK 4, Cyclin D1), and the expression of proliferation cell nuclear antigen (PCNA) were conducted. Two dayÅfs treatment resulted in selective apoptotic death of spermatogonia. The TUNEL staining analysis showed 2-BP treatment resulted in two distinct stages of morphological changes, namely the immediate effects on spermatogonia, and secondary apoptosis in spermatocytes 9 days after treatment. The expression of PCNA, a marker of proliferating cells, slightly decreased during 2-BP treatment, and it decreased significantly 9 days after treatment. CDK 2 decreased slightly during 2-BP treatment, and significantly by the day 2 or 9 after treatment, while CDK 4 significantly increased after 2-BP exposure, however, it decreased by the day 9 after exposure. Cyclin D decreased markedly during 2-BP treatment, and 2 or 9 days after treatment. Cyclin E reduced after first injection of 2-BP, and then increased a little during the second, third days injection, and then decreased again 2 or 9 days after treatment. The present study suggested that cyclin D might be involved in the apoptotic death of spermatogonia, while other CDKs might be involved in the secondary degeneration of spermatocytes.

1826 CHARACTERIZATION OF A RAT *IN VITRO* OVARIAN CULTURE SYSTEM TO STUDY THE OVARIAN TOXICANT 4-VINYLCYCLOHEXENE DIEPOXIDE.

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The heterogeneity of the mammalian ovary can cause complications for mechanistic studies involving ovarian toxicity. Repeated daily dosing of rats with the occupational chemical 4-vinylcyclohexene diepoxide (VCD) causes depletion of small preantral (primordial and small primary) ovarian follicles. These targeted populations are difficult to study due to their small size and random distribution throughout the ovary. Therefore, a rat ovarian culture system was developed and its susceptibility to VCD was assessed. Ovaries from 4-day old Fischer 344 rats were collected, laid onto membranes floating on defined medium, and covered with a drop of medium. Medium was replaced every two days. Ovaries were then prepared for histologic examination after 15 days (15d). Follicles were classified and counted in every 6th section. Initial follicle populations in ovaries consisted of primordial (80%) and small primary (20%) follicles, whereas somewhat more developed populations were present after 15d of culture (66% primordial, 32% small primary, 2% large primary, few secondary). Rat ovaries in culture for 15d were sensitive to VCD in a concentration-dependent manner. 10μM VCD had no significant effects ($p>0.05$), whereas primordial and small primary follicle populations were reduced after culturing 15d with 30μM VCD relative to controls (by 99% and 80%, respectively; $p<0.01$). To verify susceptibility of noenatal rats to VCD, rats (d4-19 of age) were dosed daily with VCD (80 mg/kg) for 15d. This resulted in a depletion of primordial (by 80%, $p<0.01$) and small primary follicles (by 70%, $p<0.05$). These results demonstrate that ovaries from neonatal rats are sensitive to VCD in culture. Because of the similarity of VCD's targets *in vitro* and *in vivo*, this system will be useful for future studies into the mechanism of action of VCD-induced ovarian follicle loss that has been well-characterized *in vivo* in rats. (ES06694, ES08979)

1827 NONYLPHENOL AND ATRAZINE INDUCE INVERSE EFFECTS ON MAMMARY GLAND DEVELOPMENT IN FEMALE RATS EXPOSED *IN UTERO*.

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These experiments tested whether exposure to 4-nonylphenol (NP) or atrazine (ATR) during fetal mammary bud outgrowth alters mammary gland (MG) development in adult female offspring. Time pregnant Long Evans rats (N=7-8/treatment) were gavaged with NP (10 or 100 mg/kg), ATR (100 mg/kg), or corn oil on gestation days (GD) 15-19. MGs from female pups were removed and prepared as whole mounts or histological sections on postnatal days (PND) 4, 22, 33 and 41. Delayed MG development was detected on PND4 in ATR pups, and persisted through PND41(stunted epithelial progression through the fat pad, fewer primary and lateral branches). High dose NP pups had advanced lobular development of their MG by PND22 (in contrast, in controls at PND41). Low dose NP glands were not morphologically different from controls. Immunohistochemical comparisons of mammary sections from PND 41 demonstrated low levels of estrogen receptor (ER) staining in control gland stroma and epithelium, and was visibly increased in tissue of pups exposed to NP and ATR. ATR also elevated ER in the stroma surrounding the terminal end buds (TEB). Progesterone receptor (PR) staining was visibly decreased in the epithelium of NP100 glands *vs.* control glands. However, PR was present at high levels in epithelium of NP10 glands and was even more prominent in ATR-exposed ductal epithelium and fat cell nuclei. PR was expressed in TEB of all rats, but was undetectable in the TEB cap cell layer. Prolactin staining was elevated *vs.* control levels only in glands containing lobule areas (NP-exposed). These qualitative evaluations suggest that the environmental contaminants, NP and ATR, have opposite effects on MG development following gestational exposure. These exposures during a critical period of epithelial outgrowth altered levels of mammary PR and prolactin, which may contribute to the differences in mammary morphology at PND41. (This abstract has been reviewed in accordance with USEPA policy and approved for publication)

1828 ACTIVATION OF MITOGEN-ACTIVATED PROTEIN KINASES AND AP-1 TRANSCRIPTION FACTOR IN OVOTOXICITY INDUCED BY 4-VINYLCYCLOHEXENE DIEPOXIDE IN RATS.

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Previous studies indicated that ovotoxicity induced in rats by 4-vinylcyclohexene diepoxide(VCD) is *via* acceleration of the normal rate of atresia. Mitogen-activated

protein kinases are thought to play a pivotal role in transmitting transmembrane signals required for cell proliferation or death. This study was designed to investigate whether the MAPK signaling pathways were involved in VCD-induced ovotoxicity. Female F344 rats were dosed daily with VCD (80 mg/kg, i.p.) for 1 day (1d, ovotoxicity has not been initiated), 10 days (10d; early signs of impending follicular destruction with no follicle loss) or 15 days (15d; significant follicle loss underway). 4 h following the final dose, ovaries and livers were collected. Ovarian small pre-antral follicles (25-100 μm) were isolated, and sub-cellular fractions were prepared. Activities of MAPKs, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal protein kinase (JNK), and p38 kinase, were determined in cytosolic fractions. Activator protein 1 (AP-1) DNA-binding activity was determined in nuclear fractions. The data obtained from ovarian small pre-antral follicles are summarized in the following table (* $p<0.05$; ** $p<0.01$ compared with control). VCD did not affect any these measurements in liver. Taken together, these data support that the acceleration of atresia is associated with the activation of JNK and p38 kinases and reduced AP-1 DNA binding activity after VCD-induced follicle destruction is underway. This kinase activation or inactivation may be crucial in mediating the normal rate of *atresia* in small pre-antral follicles. (ES09246; ES06694; HD38955).

	1d (VCD/Control)	10d (VCD/Control)	15d (VCD/Control)
ERK	1.12±0.14	0.96±0.09	1.02±0.21
p38	0.92±0.08	1.64±0.14**	1.48±0.11**
JNK	0.86±0.1*	1.44±0.11**	1.37±0.06**
AP-1	1.21±0.21*	0.29±0.04**	0.51±0.04**

1829 STRAIN COMPARISON OF ENDOCRINE RESPONSE IN RATS TO BROMODICHLOROMETHANE (BDCM) DURING PREGNANCY.

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Previously, we reported that BDCM, a drinking water disinfection by-product, causes pregnancy loss, i.e. full-litter resorption (FLR) in F344 rats when treated during the luteinizing hormone (LH)-dependent period. We also found that Sprague-Dawley (SD) rats were unaffected at even higher doses. Here, we examined the effects of BDCM on serum progesterone and LH levels during pregnancy in both strains. BDCM, in 10% emulphor, was dosed by gavage on gestational days (GD) 6-10 (plug day = GD 0) at 75 mg/kg/d in the F344 rat, and 100 or 200 mg/kg/d in the SD rat. Blood samples were collected from the lateral tail vein once daily on GD 6-11; serum progesterone and LH were measured using DELFIA®. The rats were allowed to deliver and pups were examined postnatally. FLR was confirmed by staining uterine resorption sites with 10% ammonium sulfide. Unlike the F344 rats, the SD rats maintained their litters and progesterone levels; although, similar to the F344 rats, they did display decreased LH levels. F344 rats had an 88% (7/8) incidence of FLR at 75 mg/kg/d, whereas SD rats had 0% (0/10, 0/9) incidences at both 100 and 200 mg/kg/d. BDCM-treated F344 dams with FLR had reduced progesterone and LH levels on GD 10; mean ± SE LH levels were 0.07 ± 0.04 *vs.* 0.15 ± 0.02 ng/ml for controls. In contrast, the SD rats treated at 200 mg/kg/d did not show a decrease in serum progesterone levels on GD10; however, serum LH levels were significantly decreased from controls (0.24 ± 0.06 *vs.* 0.49 ± 0.06 ng/ml). Importantly, control serum LH values were significantly higher in SD than F344 rats ($p<0.0001$). Thus, these dramatic strain differences in baseline LH levels and susceptibility to BDCM-induced pregnancy loss exemplify the importance of strain as a consideration when assessing reproductive toxicity. [Funded by the EPA/UNC Toxicology Research Program, Training Agreement CT827206 Curriculum in Toxicology, UNC-Chapel Hill. This abstract does not necessarily reflect EPA policy.]

1830 A STUDY TO EVALUATE THE EFFECTS OF LEPTIN ADMINISTERED SUBCUTANEOUSLY DURING CRITICAL PHASES OF GESTATION IN MICE.

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Leptin is a protein hormone, produced by adipose tissue, involved in the regulation of body fat. Recombinant r-metHuLeptin (Leptin) administered to normal animals results in rapid loss of body weight primarily due to body fat reduction. Previous studies with Leptin administration continuing into late gestation resulted in reduced postnatal pup survival. The objective of this study was to evaluate if the effects of Leptin previously noted are related to a critical phase of fetal development. For this study, Leptin, 10 mg/kg/day, was administered daily on Gestation Days (GD) 6-15, 15-18, or 6-18. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. Laparohysterectomies were done on GD 18 for one subset of females from each treatment regimen. Another subset was allowed to deliver and rear the offspring to Lactation Day 21. Increased incidences of prolonged gestation and/or dystocia and increase in length of gestation were noted in the groups that were administered Leptin GD 6–18 or 15–18. Body weight reductions typical of Leptin administration were noted in all Leptin-treated groups. Food consumption was reduced GD 15-18 for females treated during that period. Changes in clinical chemistry parameters were typical of previous studies conducted with Leptin. At laparohysterectomies, no changes in intrauterine growth and survival were noted and there were no treatment-related external malformation or variations observed in the fetuses. Mean live litter size and postnatal pup survival were reduced in the groups that were administered Leptin GD 6–18 or 15–18. Mean pup body weights were reduced in the groups that were administered Leptin GD 6–18. The effects of Leptin administration were considered to be most prevalent when the dams were treated late in gestation (GD 15–18), confounding the process of parturition and resulting in higher mortality rate in the F1 pups and decrements in postnatal growth.

1831 TISSUE- AND STRAIN-SPECIFC EXPRESSION OF MULTIDRUG RESISTANCE PROTEIN 1 (MRP1) IN INBRED MOUSE STRAINS.

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MRP1 is a member of the ATP-binding cassette (ABC) superfamily of membrane-spanning transporter proteins. These proteins catalyze the ATP-dependent export of many hydrophobic compounds and products of Phase II metabolism, including steroid hormone conjugates, across cellular membranes, and are implicated in the acquired resistance of tumors to chemotherapeutic agents. The purpose of this work was to compare constitutive expression levels of MRP1 in five commonly used strains of female laboratory mice. MRP1 protein was detected in tissue membranes by Western blot analysis. In all strains of mice examined, the highest expression of MRP1 was observed in the reproductive tract, particularly the ovary and fallopian tube. Marked strain differences were noted in the uterus as expression was highest in 129Sv ∽ C57BL/6 > CD-1 > B6C3F1 ∽ FVB. Strain differences were also noted in skeletal muscle and kidney; in muscle, levels of MRP1 were highest in B6C3F1 ∽ FVB ∽ CD-1 > C57BL/6 > 129Sv, whereas, in kidney, MRP1 levels were highest in C57BL/6 ∽ 129Sv > CD-1 > B6C3F1 ∽ FVB. Examination of MRP1 mRNA in tissues of B6C3F1 mice using branched DNA methods revealed that mRNA levels are not consistent with protein expression, as mRNA levels in ovary were not markedly different from other tissues. These results suggest that the regulation of MRP1 expression in mice is: (1) highly complex; (2) under the control of several factors that may be functionally polymorphic among mouse strains and (3) may contribute to phenotypic differences in xenobiotic metabolism and distribution. In addition, the abundance of MRP1 in the ovary coupled with its ability to transport conjugates of estrogen and other steroids, suggest an important role for this protein in the reproductive tract and implicates it in mechanisms of reproductive toxicology.

1832 EFFECTS ON THE GONADAL DEVELOPMENT OF MEDAKA (*ORYZIAS LATIPES*) EXPOSED AS EMBRYOS TO BROMODICHLOROMETHANE .

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The purpose of this study is to determine the toxic effects of bromodichloromethane (BDCM) on the developing gonad of medaka. BDCM is one of the trihalomethanes, a disinfection by-product found in the drinking water with developmental/reproductive toxicity concerns expressed in epidemiological studies. Medaka embryos at early high blastula stage were exposed to 10 or 25 mg/L BDCM for 10 days, and transferred to contaminant-free embryo rearing medium until hatched. Upon hatching, medaka fry were grown-out in spring water for 6 and 12 months. Two controls (embryo-rearing solution and DMSO carrier) were used. Fish were sampled at 6 and 12-month post-exposure and processed for histological evaluation. The evaluated endpoints, indicative of endocrine disruption or other reproductive toxicities were: hermaphroditism; immature gonads; ectopic gonads; male:female ratio, and other pathology. Hermaphroditism was not observed. There were two cases of ectopic gonads (10 mg/L BDCM and DMSO exposure groups) in the female medaka, but none in male medaka. The male: female ratios were skewed towards males suggesting possible androgenic or anti-estrogenic effects

of BDCM. Exposure to BDCM resulted in a dose-dependent increase in the proportion of males. Immature testis and ovaries, found in all concentrations including controls, are under evaluation. Other pathologic observations were granulomas and cystic ovaries, and granulomas in the testis with no apparent differences between exposed and control groups. The medaka embryo exposure protocol is useful for screening for endocrine disruption/reproductive toxicity. (Abstract may not reflect USEPA policy. The opinions expressed in this abstract are those of the authors and not necessarily those of EPA. Funded in part by USEPA and DOD Cooperative Agreement)

1833 OVEREXPRESSION OF INSULIN-LIKE GROWTH FACTOR (IGF)-I WITH IGF-I RECEPTOR SIGNALING AND MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) ACTIVATION IN HUMAN UTERINE LEIOMYOMAS.

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Uterine leiomyomas (fibroids) are benign tumors that are clinically apparent in up to 25-30% of women over 30 years of age in the USA. Although the cause of fibroids remains unknown, research in our laboratory suggests that the cell signaling pathways activated by the IGF-I peptide may be important in tumor growth during the proliferative phase of the menstrual cycle. IGF-I can bind with either IGF-I receptor (IGF-IR) or insulin receptor (IR) and both IGF-IR and IR signaling pathways can ultimately lead to mitogen-activated protein kinase (MAPK) activation and DNA transcription, or phosphatidylinositol 3-kinase activation and possibly cell survival. In this study, we examined leiomyoma and normal myometrial tissue from premenopausal women. IGF-I, IGF-IRβ, IR, and phospho-p44/42 MAPK were immunolocalized in tissue samples. Activation of the IGF-IR signaling pathway was assessed by identifying phosphorylated sites on the IGF-IRβ subunit and phosphorylation of the p44/42 active sites of the downstream effector MAPK using Western blot analysis. We found that IGF-I was increased and concentrated in fibrous stromal regions and smooth muscle cells of leiomyomas. However, in myometrial samples, staining was mostly perivascular in localization and minimal. Both leiomyoma and myometrial samples expressed IGF-IRβ; however, greater phosphorylation of IGF-IRβ was observed in the tumors compared to myometrium. IR was only minimally present in both leiomyomas and myometrial samples, and therefore most likely not a major signaling pathway utilized by IGF-I in these tumors. The downstream effector protein MAPK was expressed more intensely in the nuclei of leiomyoma cells than in the smooth muscle cells of the myometrium. These data show that the IGF-I peptide is overexpressed in uterine leiomyomas compared to myometrium, and that the IGF-IR signaling pathway and activation of MAPK may be important in the growth of uterine leiomyomas.

1834 EFFECTS OF POLYVINYL ALCOHOL ADMINISTERED IN THE DIET TO RATS ON FERTILITY, EARLY EMBRYONIC DEVELOPMENT, GROWTH AND DEVELOPMENT.

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Polyvinyl Alcohol (PVA) is a water soluble synthetic polymer prepared from the polymerization of vinyl acetate and the subsequent partial hydrolysis of polyvinyl acetate. PVA is used as a polymer for film coating pharmaceutical and dietary supplement tablets where moisture barrier/protection properties are required. PVA was administered in the diet to rats (26/sex/group) at treatment levels of 0, 2000, 3500 and 5000 mg/kg/day for 2 generations. The study design assessed gonadal function, estrous cycle, mating behavior, conception, gestation, parturition, lactation, weaning, and growth and development of F_1 and F_2 offspring. Parental rats were treated for 70 days prior to mating, throughout mating, gestation and lactation until sacrifice. Clinical observations, body weights and feed consumption were routinely recorded. Dietary concentrations were adjusted for each sex to provide the intended mg/kg/day PVA levels on a weekly basis except during gestation and lactation. Pups were weighed routinely and weaned at 21 days of age prior to selection for the next generation. Unformed stool was noted predominately at the 3500 and 5000 mg/kg/day levels in P_0 and F_1 parental animals. This finding was attributed to the high levels of PVA being fed and subsequently excreted in the stool. Slight decreases in the mean body weights of P_0 males were noted at 2000 and 5000 mg/kg/day. Feed consumption was elevated at the 3500 and 5000 mg/kg/day treatment levels in both generations but not during either lactation period. These increases generally were observed in a dose-related manner (grams/kg/day), as a result of the large amount of PVA being consumed to maintain the caloric intake necessary for normal growth. There were no effects of PVA on P_0 or F_1 male or female reproductive performance or pup survival, growth, organ weights, and macroscopic or microscopic observations at dietary concentrations of 2000, 3500 and 5000 mg/kg/day.

1835 METABOLIZING ENZYMES IN THE RAT EPIDIDYMIS AND EFFERENT DUCTS: POTENTIAL ROLE IN MALE REPRODUCTIVE TOXICITY.

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The presence of metabolizing enzymes in the epididymis and efferent ducts may indicate that these structures are potential toxicologic targets *via* metabolic activation. Previously, we proposed that the epididymis may be toxicologically analogous to the kidney. Our studies have focused on identifying and localizing enzymes involved in nephrotoxic responses, such as cytochrome P4502E1, soluble epoxide hydrolase, and cysteine conjugate β-lyase (β-lyase). We immunolocalized P4502E1 in the efferent ducts, and confirmed those findings with Western blotting and enzyme activity assays. Likewise, soluble epoxide hydrolase was localized in the efferent ducts, epididymis, and epididymal sperm. Our most recent studies provide evidence for the presence of β-lyase in the excurrent duct system. Using immersion-fixed frozen embedded tissues from untreated rats, β-lyase was immunolocalized in 10μm sections probed with anti-rat β-lyase (gift of James L. Stevens) and AlexaFluor488 conjugated IgG (Molecular Probes). Preliminary fluorescent imaging indicated a clearly visible, diffuse signal in the efferent ducts when compared to kidney controls. The caput, corpus, and cauda epididymis were slightly reactive, while the initial segment was devoid of any antibody reactivity. To confirm these findings, kidney and excurrent duct proteins were separated on a 10% SDS gel, affixed to nitrocellulose, and probed with the same antibody. Preliminary immunoblot data indicated the presence of strongly reacting band (~55kDa) in kidney cytosol and mitochondria. A similar-sized band was present in epididymal and efferent duct cytosolic fractions, as well as in the efferent duct S9 pellet. These bands were not present in similar membranes immunoblotted without antibody. Overall, our data indicate that enzymes associated with nephrotoxicity are concentrated to a higher degree in the efferent ducts than in the epididymis. Such findings suggest that the proximal excurrent ducts are a potential target for compounds typically considered nephrotoxicants, such as chlorinated solvents.

1836 IN-LIFE EFFECTS OF DAILY CHLORDANE EXPOSURE AT ENVIRONMENTAL LEVELS IN F0 RATS AND THEIR NEONATES.

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This in-life study of FO-rats and their neonates was a part of Replicate II of a multigenerational investigation to evaluate the effects of technical chlordane. Chlordane, a formerly used pesticide and currently an ATSDR priority-listed hazardous substance, is a bioaccumulative and persistent organochlorine pollutant in the environment. The in-life pregnancy and neonatal effects in humans and other mammals exposed to environmental levels of chlordane used in this study are unknown. Sprague-Dawley FO-rats were gavage-fed daily with chlordane at 0.0, 0.125, 0.25 and 1.0 mg/kg body weight. In-life measurements included body weight, feed intake, mating index, gestation length and weight, parturition index, litter size and weight, pup mortality at birth, sex ratio and ano-genital distance. Chlordane treatment did not affect parental body weight and feed intake, mating index, gestation length and weight, parturition index, litter size and weight, and pup mortality at birth. However, on postnatal day one (PND1) chlordane treatment increased ($P<0.05$) male/female ratio of live pups at the 0.125 mg/kg and ano-genital distance of the male neonates at the 0.125 and 0.25 mg/kg dosed groups. The data indicated that chlordane treatment in this study elicited androgenic effects *in utero* and hence, chlordane may put humans at risk as an endocrine disruptor. (Supported by MHPF/ATSDR - #U50/ATU3922660008)

1837 THE EFFECTS OF TETRATHIOMOLYBDATE (TTM, NSC-714598) AND COPPER SUPPLEMENTATION ON FERTILITY AND EARLY EMBRYONIC DEVELOPMENT IN RATS.

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Based on its unique anti-copper action, TTM is being studied as an antiangiogenic agent and is currently in Phase II clinical trials for cancer therapy. The purpose of the present study was to evaluate the toxicity of TTM and the protection of copper supplementation on the reproductive capability of male and female CD® rats. Doses of 0, 1, 4, 12 mg/kg/day (0, 6, 24, 72 mg/m^2/day) and 12 mg/kg/day with copper supplementation (110 mg/kg of diet) were given orally by daily gavage to male rats for at least 59 days and to pregnant female rats for about 30 days, including 29 and 15 days, respectively, of dosing prior to cohabitation. There were no

drug-related toxic effects on the estrous cycle or reproductive indices, or maternal toxicity in any female rat. Therefore, the no observed effect level (NOEL) for female reproductive toxicity was at least 12 mg/kg/day (72 mg/m^2/day). Male rats given 12 mg/kg/day showed significant decreases in body weight gains and food consumption and drug-related anemia developed primarily toward the end of the study. Serum ceruloplasmin levels were dose-dependently decreased in all drug-treated male rats. At 12 mg/kg/day, rats in the copper supplemented dose group had serum ceruloplasmin levels 16 times higher than the nonsupplemented dose group. TTM given at doses of 12 mg/kg/day resulted in reduced epididymal weights, sperm counts, and sperm motility as well as sperm morphologic abnormalities including excessive sperm fragmentation and histopathologic changes in testis and epididymis. None of these drug-related effects occurred in the 1 and 4 mg/kg/day dose groups. Copper supplementation prevented the adverse sperm effects produced by 12 mg/kg/day of TTM. Therefore, the NOEL for male reproductive toxicity was 4 mg/kg/day (24 mg/m^2/day). (Supported by NCI Contract No. NO1-CM-87103.)

1838 ENHANCED MITOCHONDRIAL TESTICULAR ANTIOXIDANT CAPACITY IN GOTO-KAKIZAKI (GK) DIABETIC RATS: ROLE OF COENZYME Q.

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Since diabetes mellitus is associated with impairment of testicular function, ultimately leading to reduced fertility, this study was conducted to evaluate the existence of a cause-effect relationship between increased oxidative stress in diabetes and reduced mitochondrial antioxidant capacity. The susceptibility to oxidative stress and antioxidant capacity (in terms of glutathione, coenzyme Q and vitamin E content) of testis mitochondrial preparations isolated from Goto-Kakizaki non-insulin dependent diabetic rats and from Wistar control rats, 1 year of age, were evaluated. It was found that Goto-Kakizaki mitochondrial preparations showed a lower susceptibility to lipid peroxidation induced by ADP/Fe2+, as evaluated by oxygen consumption and reactive oxygen species generation. The decreased susceptibility to oxidative stress in diabetic rats was associated with an increase in mitochondrial glutathione and coenzyme Q9 contents, while vitamin E was not changed. These results demonstrate a higher antioxidant capacity in diabetic GK rats, that we suggest is an adaptive response of testis mitochondria to the increased oxidative damage in diabetes mellitus.

1839 THE ARYL HYDROCARBON RECEPTOR (AhR) MAY REGULATE GROWTH, BUT NOT APOPTOSIS OF ANTRAL OVARIAN FOLLICLES.

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The AhR mediates the toxicity of chemicals such as 2, 3, 7, 8 tetrachlorodibenzo-*p*-dioxin. Although little is known about the physiological role of the AhR, our previous work indicates that the AhR may alter the number of antral follicles in the ovary. Mice deficient in the AhR (AhRKO) have 54% fewer antral follicles than wild-type (WT) mice in postnatal (PN) life. The mechanism by which the AhR influences the numbers of antral follicles is not understood. It is possible that the AhR may regulate the number of antral follicles by increasing the rate of growth of immature follicles to the antral stage or by decreasing the rate of death of antral follicles. Thus, the purpose of this study was to test the hypothesis that follicles from AhRKO mice have decreased follicular growth and/or increased apoptosis compared to WT mice. AhRKO and WT (PN 28-35) mice (both on a C57Bl/6J background) were primed with pregnant mare serum gonadotropin to induce growth of immature follicles to the antral stage. After 48 hours, antral follicles were isolated from each ovary and subjected to measurements of follicular size. Additional follicles were cultured in serum free medium to induce apoptosis and then subjected to 3' end labeling to measure the amount of apoptosis. The results indicate that antral follicles from WT mice were significantly larger than antral follicles from AhRKO mice (WT = 211 ± 10 mm; AhRKO = 182 ± 6 mm; $n = 6$, $p \leq 0.02$). In contrast, antral follicles from AhRKO mice and WT mice contained similar amounts of apoptotic DNA after isolation from the ovary (AhRKO = 4619.8 ± 741.6 cpm; WT = 7843 ± 4511 cpm; $n = 3$, $p \geq 0.5$). They also contained similar amounts of apoptotic DNA after being cultured in serum free medium overnight (AhRKO = 96, 811 ± 62, 640 cpm; WT = 73, 043 ± 28, 346 cpm; $n = 3$, $p \geq 0.7$). These data suggest that the AhR may regulate the growth, but not apoptosis of antral ovarian follicles in the mouse. Supported by HD 38955 (JAF) and ES01332 (REP).

1840 EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) ON SERUM INHIBIN CONCENTRATIONS AND INHIBIN IMMUNOSTAINING DURING FOLLICULAR DEVELOPMENT IN SPRAGUE-DAWLEY RATS.

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Intact and hypophysectomized immature rats were pretreated with TCDD (0 or 32 µg/kg p.o.) and sacrificed throughout synchronized follicular development (0, 12, 24, 48 and 72 hours after equine chorionic gonadotropin, eCG). TCDD administration to intact rats resulted in a premature elevation of serum FSH and LH by 12 hours post-eCG. In intact rats pretreated with TCDD, the intensity of ovarian immunoreactivity for inhibin and the number of ovarian follicles staining for inhibin in midsaggital ovarian sections were decreased at the time of eCG administration (24 hours post-TCDD) in comparison to controls. However, this decreased ovarian staining for inhibin was not associated with alterations in serum inhibin concentrations. Serum inhibin was suppressed in comparison to intact controls only at 24 hours post-eCG (48 hours post-TCDD) in intact rats that received TCDD. Hypophysectomized animals exhibited no effect of TCDD on serum inhibin at any timepoint. In summary, TCDD reduced serum concentrations of inhibin after the premature increases in FSH and LH suggesting that inhibin is not causally related to the initial elevation of FSH following exposure to TCDD in this model.

1841 EFFECTS OF INDOLE-3-CARBINOL AND TAMOXIFEN ON OVULATION AND ITS HORMONAL REGULATION - COMPARISON TO 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD).

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Immature Sprague-dawley rats received daily doses of indole-3-carbinol (I3C, 0-1.5g/kg/day), diindolymethane (DIM, 0-400mg/kg/day), tamoxifen (TAM, 0-0.5mg/kg/day) or vehicle. Follicular development was induced by equine chorionic gonadotropin (eCG, 5 IU) one day after the initial dose. In a hormone replacement study, human chorionic gonadotropin (hCG, 10 IU s.c., 48h post eCG) was used to mimic a normal preovulatoy LH surge following treatment with either I3C or TAM. Blood and ovaries were collected thoughout follicular development and the number of ova shed was measured on the morning following normal ovulation (72 h post eCG). I3C but not TAM reduced body weight gain at higher doses after 4 days of dosing. Ovarian weight gain and ovulation were inhibited by both I3C and TAM in a dose-dependent fashion. During the preovulatory period, I3C and TAM blocked normal LH and FSH surges and supressed serum P4 profoundly without changing circulating levels of E2. After the time of expected ovulation, serum E2 was increased in rats receiving I3C or tamoxifen, whereas serum P4 was decreased dose dependently. DIM exerted no significant effects on any of the endpoints studied even at the highest dose. hCG restored ovarian weight gain and ovulation in TAM-treated rats. However, hCG only partially reversed the blockage of ovulation by I3C, although ovarian weight gain was restored to normal. In summary, I3C seems to block ovulation by mechanism(s) similar to TCDD (at both ovarian and hypothalamic levels), whereas TAM appears to alter ovulation primarily *via* effects at the hypothalamic-pituitary axis.

1842 MULTI-GENERATION REPRODUCTION TOXICITY STUDY OF 3, 3', 4, 4'-TETRACHLOROAZOBENZENE ADMINISTERED TO SPRAGUE-DAWLEY RATS BY ORAL GAVAGE.

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3, 3', 4, 4'-Tetrachloroazobenzene (TCAB) is generated as a by-product during the manufacture or environmental degradation of 3, 4-dichloroaniline and its herbicidal derivatives. TCAB was tested for reproductive toxicity in Sprague-Dawley rats

using the NTP Reproductive Assay by Continuous Breeding protocol. F_0 males and females (20/sex/group) were administered TCAB by corn oil gavage at concentrations of 0, 1, 3, and 10 mg/kg/day. Mating pairs produced 3 F_1 litters/pair and the 3rd litter was reared to adulthood. F_1 weanlings were exposed to TCAB at the same concentrations as their F_0 parents and on postnatal day 81± 10, 20 pairs of F_1 adults/dose were mated to produce 3 F_2 litters/pair. Parental observations included body weight, feed consumption, organ weights, and gross and microscopic pathology. Litter observations included number of live pups/litter and live pup weight. F_0 parental body weights were decreased minimally during the study. There were significant reductions in the number of live F_1 pups/litter in all dose groups. Mean body weight was decreased significantly in F_1 parents treated with 10 mg/kg. The number of live F_2 pups/litter was significantly reduced at 3 and 10 mg/kg and live pup weight was decreased at 10 mg/kg. A crossover mating was conducted with the F_0 parents to determine which sex was affected by TCAB treatment. Treated F_0 males and females from the control and 10 mg/kg groups were paired with naïve males and females for 7 days. The reproductive data for the treated males was comparable to the controls. When treated females were cohabited with naïve males, the number of live males/litter was decreased by 53%. There was no effect on the number of primordial follicles in the ovaries of F_0 females treated with 10 mg/kg TCAB relative to controls suggesting that *in utero* developmental toxicity was responsible for the reductions in litter size.

1843 EFFECTS OF GESTATIONAL AND LACTATIONAL EXPOSURE TO GENISTEIN ON SPERM QUALITY AND TESTICULAR GENE EXPRESSION IN MICE.

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The objective of this study was to examine the effects of gestational and lactational exposure to phytoestrogen genistein on sperm quality, *in vitro* sperm fertilizing ability and testicular gene expression. Pregnant mice were gavaged with 0.1, 0.5, 2.5 or 10 mg/kg genistein in corn oil from gestational day (GD) 12 to postnatal day (PND) 21. Anogenital distance (AGD) of male offspring was recorded on PND 7 and PND 21. On PND 21, 105 and 315, body weight and testis weight were recorded. On PND 105 and 315, sperm count and sperm fertilizing ability of F1 males were assessed *in vitro*. Testicular gene expression was examined using a custom mouse cDNA microarray representing 2689 cDNA. Testicular RNA from mice in the 10 mg/kg dose group on PND 21 and 105 was compared to that from corresponding control mice. Testis weight was slightly increased in the 10 mg/kg group at PND 105 and 315 but not significantly ($p>0.05$). AGD was decreased on PND 21 in the 10 mg/kg dose group ($p<0.05$). No effect on sperm motility or sperm count was observed. *in vitro* fertilizing ability was significantly increased on both PND 105 (18.7%, $p<0.001$) and PND 315 (17.7%, $p<0.001$) in the 10 mg/kg group, but it was decreased by 8.5% on PND 105 in the 0.5 mg/kg dose group ($p<0.05$). The percent of fragmented eggs was decreased significantly on PND 105 in the 0.1, 2.5 and 10 mg/kg dose groups ($p<0.001$) and slightly increased on PND 315 10 mg/kg dose group. The percentage of one-cell fertilized eggs was significantly decreased on PND 315 in the 0.5, 2.5 and 10 mg/kg dose groups ($p<0.001$). Microarray data were analyzed using paired t-tests to identify differentially expressed genes. Five genes were under-expressed on PND 105, but there were no changes on PND 21. This study suggests that exposure to genistein in early development increases sperm fertilizing ability in adult mice.

1844 NITROBENZENE-INDUCED TESTICULAR GERM CELL APOPTOSIS IN YOUNG AND ADULT FASL-DEFICIENT (*GLD*) MICE.

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Apoptosis of testicular germ cells (GCs) is essential for functional spermatogenesis. We have previously shown that the Fas/CD95 signaling system is important for triggering GC apoptosis in four-week old male C57/bl6J (C57) mice testes after MEHP (mono-2-ethylhexyl phthalate, 1g/kg p.o.)-induced Sertoli cell injury. However, in B6Smn.C3H-Fasl-*gld* (*gld*) mice, that express a nonfunctional form of Fas Ligand, a profound reduction in MEHP-induced GC apoptosis was observed as compared to C57 mice. To test if Fas-mediated GC apoptosis is a common signaling event after Sertoli cell injury, we evaluated the Sertoli cell toxicant, nitrobenzene (NB). Four-week old C57 and *gld* male mice were dosed with NB (600mg/kg p.o.) and testes were collected at 0, 16, 20, 24 hours. Apoptosis was quantified by determining the percentage of seminiferous tubules that contain >3 TUNEL positive germ cells. Vehicle (corn oil) treated 4 week-old C57 and *gld* mice exhibited less than 10% germ cell apoptosis. After NB exposure, both C57 and *gld* mice showed variable (10-65%) increases in GC apoptosis, with a trend for larger increases in the C57 mice at 16 h, whereas similar increases occurred in the testes of *gld* mice at the later (20 & 24h) time points. Eight-week old C57 and *gld* male mice were dosed with NB (800mg/kg p.o.) and testes were collected at 0, 3, 6, 12, 24 hours. Vehicle-treated C57 and *gld* mice both showed ~4% apoptosis. As opposed to the young mice, the levels of GC apoptosis remained at control levels for 3, 6, and 12 hours post NB exposure. However, a large increase in GC apoptosis (C57: 50.36 ± 3.11%; *gld*: 66.10 ±2.18%, mean ± SD) occurred after 24 hour after NB treatment. Taken together, these findings suggest that a FasL-independent signaling mechanism may be responsible for NB-induced germ cell apoptosis in both young and adult C57 and *gld* mice. (Supported by RO1ES09145, NSF's LSAMP, and UT Co-op Society).

1845 EFFECTS OF GESTATIONAL AND LACTATIONAL EXPOSURE TO GENISTEIN ON OVULATION AND EGG FERTILIZING ABILITY IN MICE .

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Genistein (GEN) is an isoflavonold phytoestrogen in soybeans. Our previous study demonstrated that gestational and lactational exposure to diethylstilbestrol decreases the number of eggs produced in superovulated mature female mice, without affecting egg-fertilizing ability. The objective of the study was to examine the effect of gestational and lactational exposure to GEN on ovulation and egg fertilizing ability in female offspring. Pregnant C57BL/6 mice (F-0) bred with DBA/2 male were gavaged with 0, 0.1, 0.5, 2.5, or 10 mg GEN/kg of body weight/day in 0.1 ml corn oil from gestational day 12 to postnatal day (PND) 21. No significant effects on fecundity ($P>.1$) or 4- and 21-day pup survival were observed. An increase in litter size in the 0.5 mg treatment group was observed ($P<.05$). There were no effects on sex ratio, litter weight, or average birth weight of pups among treatment groups ($P>.05$). AGD of the female offspring (F-1) in 2.5 mg and 10 mg treatment groups were longer than that in the controls ($P<.05$). In 3-6 week old F-1 females, the number of eggs ovulated was not different among treatment groups, In 8-11 week old females, a trend of decrease in the number of eggs ovulated was observed in the 0.5 mg treatment group ($P=.09$). Fertilizing ability of eggs from 3-6 week old females in the 0.1mg/kg treatment group was significantly lower than that of controls ($P<.05$), but no effect on the eggs from the 8-11 week old females was observed. Results from this study suggest that exposure to low dose of GEN during gestation and lactation decreases fertilizing ability of eggs from pre-pubertal F-1 females, while high doses of GEN exposure increase the number of non-viable eggs in the mature F-1 females. (This work is supported, in part, by EPA, R827-420-01-0.)

1846 METHOXYCHLOR MAY DAMAGE THE MURINE OVARY AND VAGINA.

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Methoxychlor (MXC) is an organochlorine pesticide that is currently used to protect agricultural products from insects. Although this chemical is a suspected reproductive toxicant, its effects on the ovary and vagina are not well understood. Thus, the purpose of this study was to test whether MXC induces ovarian atresia and vaginal keratinization by direct action on the ovary and vagina or by indirect action involving the disruption of hormone levels. To test this hypothesis, adult CD-1 mice were dosed with sesame oil (control) or MXC (8, 16, 32 mg/kg/d) for 10 or 20 days. Estrous cyclicity and vaginal keratinization were evaluated daily *via* vaginal lavage. After dosing, ovaries were collected for histological evaluation of follicular atresia and blood was collected for measurement of follicle-stimulating hormone (FSH) levels and estradiol (E_2) levels. The results indicate that MXC disrupted estrous cyclicity (controls = 33% abnormal cycles, n=27; 8mg/kg MXC = 56% abnormal cycles, n=16; 16 and 32mg/kg MXC = 60% abnormal cycles; n=10 for each dose) and induced vaginal keratinization. In addition, MXC increased the amount of follicular atresia compared to controls (controls = 52.9% ± 3.6 atretic follicles, n=9; 32mg/kg MXC = 71.8% ± 1.5 atretic follicles, n=4; $p < 0.05$). MXC did not alter hormone levels at any dose. After 10 days of dosing, FSH levels were 4.6 ± 0.8ng/ml in controls and 3.4 ± 0.3ng/ml in mice treated with 32mg/kg MXC ($p\geq0.5$; n=3). E_2 levels were 148.9 ± 27.3pg/ml in controls and 121 ± 14.7pg/ml in mice treated with 32mg/kg MXC ($p\geq0.8$; n=3). These data suggest that MXC disrupts estrous cyclicity, induces vaginal keratinization, and causes follicular atresia by a direct action on the ovary and vagina and not by an indirect action involving FSH and E_2. Supported by HD 38995, T32-ES07263, and the Women's Health Research Group at the University of Maryland.

1847 NITRIC OXIDE DECREASES UTERINE CONTRACTILITY BY BLOCKING THE CLOSURE OF ATP-DEPENDENT POTASSIUM CHANNELS.

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Both nitric oxide and ATP-dependent potassium channel activators decrease uterine tone and inhibit spontaneous rat and human myometrial contractions during pregnancy. We tested the hypothesis that nitric oxide hyperpolarizes myometrial cells, cultured from gestation day 20 rats, by opening ATP-dependent potassium channels. Functional activity of ATP-dependent potassium channels was measured by evaluating changes in membrane potential using the bis-oxonol dye DiBAC4(3) and confocal microscopy. As indicated by a reduction in fluorescence intensity from pretreatment, ATP-dependent potassium channel opener pinacidil (1 μM) hyperpolarized myometrial cells and increased significantly their membrane potential compared with solvent controls. Glibenclamide (5 μM), an ATP-dependent potassium channel blocker, caused a significant depolarization, indicated by increased fluorescence, followed by hyperpolarization. Pretreatment with the nitric oxide donating compound spermine NONOate (25 μM) inhibited the observed depolarization and augmented the hyperpolarization state caused by glibenclamide. Carboxy-PTIO, a nitric oxide scavenger, prevented the inhibitory effects of spermine NONOate on the glibenclamide-induced hyperpolarization. A role for ATP-dependent potassium channels in nitric oxide's effects on contraction frequency is suggested by the observation that treatment of uterine strips with glibenclamide blocked relaxation by spermine NONOate. Previous findings support an inhibitory function for oxidative stress. Collectively, these data suggest an equilibrium between oxidative stress and nitric oxide in the maintenance of potassium channels and uterine tone.

1848 HUMAN TISSUE MODEL FOR VAGINAL IRRITATION STUDIES.

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Recently, a tissue culture based model of the vaginal epithelium has been developed. Normal, human ectocervico-vaginal (ECV) epithelial cells were induced to form a three-dimensional tissue using specially formulated serum free medium. The *in vitro* tissue reproduces many of the histological, ultrastructural, and protein expression properties of native tissue, including inter-digitation of cells, glycogen production, and cytokeratin expression. Initial experiments investigated the use of this tissue model and the MTT tissue viability assay for predicting ECV irritation. Vaginal anti-fungal products, contraceptives and lubricants were exposed to the tissue model and the exposure time which causes a 50% reduction in tissue viability (ET-50) was determined. The ET-50s were compared to rabbit vaginal irritation scores for 7 products which are currently marketed or in clinical trials. Using an ET-50 cutoff of 9.0 hrs, the products could be successfully categorized into minimal or mild irritation classes. Based on these results and the problems associated with obtaining and the handling of human vaginal tissue, it is anticipated that the tissue model will be very useful in assisting product development scientists in developing safe, efficacious products.

1849 EFFECTS OF DIETARY ETHINYL ESTRADIOL (EE2) ON THE MAMMARY GLAND OF MALE CD (SPRAGUE-DAWLEY) RATS.

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Previous dose range-finding experiments had indicated that EE2 and the soy phytoestrogen genistein induced hyperplasia in pubertal (PND 50) male mammary glands and that this was among the most sensitive indicators of activity of these compounds. The present study was designed as an adjunct to a subsequent multigeneration bioassay to determine if this effect was reproducible, required continuous dosing, and persisted into adulthood. The parental generation received a soy- and alfalfa-free diet containing 0, 2, 10, or 50 ppb EE2 starting 28 days prior to mating. Dosing continued throughout pregnancy and lactation. F_1 pups were fed the same diet as their parents until sacrifice. One male from each of 18 litters was sacrificed at PND 50 and another at PND 90. F_2 pups were removed from dose at weaning (PND21) and sacrificed according to the same schedule as the F_1 pups. Mammary glands were removed, fixed in 10% NBF, and processed for microscopic evaluation. Hyperplasia of the mammary gland in the F_1 rats was evident at PND 50 and PND 90. Mostly mammary ducts were affected at PND 50 (control 2/18, 2 ppb 4/18, 10 ppb 6/18, 50 ppb 14/18), while both ducts and alveoli were hyperplastic in dose dependent-manner at PND 90. In the F_2 generation, there was still a trend of ductal hyperplasia at PND 50 (control 3/18, 2 ppb 4/18, 10 ppb 6/18, 50 ppb 7/17), but by PND 90 treatment groups were similar to control. We conclude that hyperplasia of mammary gland in male rats is a sensitive indicator of estrogenic activity of EE2, and perhaps other estrogens, but appears to require continuous exposure.

1850 EFFECT OF DIETARY ETHINYL ESTRADIOL (EE2) ON SERUM TESTOSTERONE, PROSTATE WEIGHT, AND ESTROGEN RECEPTORS (ER) IN CD (SPRAGUE-DAWLEY) RATS.

B. Blaydes[1], C. Bryant[1], R. R. Newbold[2] and K. B. Delclos[1]. *[1]NCTR, Jefferson, AR and [2]NIEHS, Research Triangle Park, NC.*

Estrogen treatment of neonatal rodents has been shown to induce permanent effects on the prostate, but the dose levels at which such permanent effects can be elicited have sparked controversy in recent years. Recently, subclinical doses of EE2 have been reported to effect the reproductive tract in male mice (Thayer et al., Human Reprod., 16, 988, 2001). In a dose range-finding study with dietary EE2 in Sprague-Dawley rats, we had found a marginally increased dorsolateral prostate weight in animals sacrificed at PND 50 in the intermediate dose range (5 ppb, approximately 1 μg/kg body weight/day). The results reported here were obtained in a subsequent multigeneration study using doses of 0, 2, 10, and 50 ppb EE2 administered in a soy- and alfalfa-free diet. Dosing was begun for the parental generation 28 days prior to mating and was continued throughout pregnancy and lactation. F_1 pups were fed the same diet as their parents continuously. One male from each of 18 litters was sacrificed at PND 50 and another at PND 90. F_2 pups were removed from dose at weaning (PND21) and sacrificed according to the same schedule as the F_1 pups. Serum testosterone levels were significantly depressed in PND 50 animals of the F_1 (10 and 50 ppb) and F_2 (50 ppb) generations. However, no significant effects on ventral or dorsolateral prostate weights were observed in F_1 or F_2 animals at PND 50 or PND 90. Prostates from 12 animals per treatment group were analyzed by Western blot for levels of ERs α and β. Subtle treatment-related changes, not greater than 2-fold, were observed in both prostate lobes, with the most pronounced change being a decreased level of ERα in the PND 90 ventral prostate. These results indicate that while low doses of EE2 have demonstrable effects on serum testosterone and prostatic estrogen receptors in the male Sprague-Dawley rat, the effect on testosterone appears to be transient and prostate weight is not a robust indicator of these effects under the experimental conditions used here.

1851 EFFECTS OF EXPOSURE TO VINCLOZOLIN ADMINISTERED IN A SOY- AND ALFALFA-FREE DIET ON MALE AND FEMALE CD (SPRAGUE-DAWLEY) RATS.

C. Weis[1], J. R. Latendresse[2], T. J. Bucci[2], R. R. Newbold[3] and K. B. Delclos[1]. *[1]NCTR, Jefferson, AR, [2]PAI, Jefferson, AR and [3]NIEHS, Research Triangle Park, NC.*

Developmental exposure of male pups to the fungicide vinclozolin induces a spectrum of effects that parallel those of the androgen receptor antagonist flutamide. There is a discrepancy in the doses at which some of these effects have been observed (Gray et al., Toxicol. Ind. Health, 15: 48, 1999; Hellwig et al., Reg. Toxicol. Pharmacology, 32: 42, 2000). Our laboratory is conducting a series of studies of endocrine active compounds, including vinclozolin, administered in a soy- and alfalfa-free diet that has low and carefully monitored levels of the phytoestrogens genistein and daidzein. In the present study, the compound was administered at dose levels of 0, 2, 10, 50, 150, 300, and 750 ppm to dams from gestation day 7 through delivery and lactation. Pups (5 litters, standardized to 4 per sex) were continued on the same dosed feed as their parents until sacrifice at PND 50. Ingested doses ranged from approximately 0.2 – 85 mg/kg body weight/day Gestational duration, total and live pups per litter, litter sex ratios, and birth weight were not significantly affected, nor were body weight and food intake for dams or pups. Anogenital distance was decreased only in male pups at the highest dose. Preputial separation was delayed, seminal vesicle/coagulating gland weight decreased, and female liver weight increased at 150 ppm and above. Atrophy of the seminal vesicles was noted microscopically only at 750 ppm. In the 10 ppm dose group (approximately 1 mg/kg/day), vaginal opening was significantly delayed and absolute dorsolateral prostate and epididymal weights were increased. When analyzed with body weight as a covariate, the epididymal weight was also elevated at 10 ppm and dorsolateral prostate weight showed a significant quadratic dose trend. These latter results are unexpected and require replication. If replicated, further investigation would be required to explain these effects since they are not consistent with the known antiandrogenic action of vinclozolin.

1852 GENETIC SUSCEPTIBILITY TO INHALED POLLUTANTS.

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A person's susceptibility to inhaled pollutants is invariably multi-factorial, depending on the individual's age, gender, socioeconomic and ethnic background, and many other variables. However, there is increasing interest in understanding the susceptibility or resistance of individuals to environmental agents based on genetic differences. It is important to understand how genetic polymorphisms in the general population affect responses to environmental agents in order to correctly interpret inter-individual differences in toxicological studies and to ultimately improve risk assessment and environmental policies. This symposium will present current technologies and experimental approaches to identify potential genetic loci regulating susceptibility to inhaled pollutants. The symposium will present evidence from human familial inheritance studies and linkage analysis from inbred mouse strains on the susceptibility to environmental lung diseases induced by inhalation of particles or gases. In addition, the presentations will address state-of-the-art technology, such as novel high-throughput single nucleotide polymorphisms (SNP)-based genotyping method, which could greatly accelerate the identification of potential susceptibility genes. The presenters will demonstrate that inter-individual differences in responses often observed in toxicological studies may be strongly linked to genetic determinants.

1853 HUMAN STUDIES IN GENETIC SUSCEPTIBILITY.

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Inter-individual variation in human responses to air pollutants suggests that some subpopulations are at increased risk to the detrimental effects of pollutant exposure. Extrinsic factors such as previous exposure and nutritional status may influence individual susceptibility. Intrinsic (host) factors that determine susceptibility include age, gender, and pre-existing disease (e.g. asthma), and it is becoming increasingly clear that genetic background also contributes to individual susceptibility. Environmental exposures and genetic factors associated with disease risk interact in a complex fashion that varies from one population to another. The relationships between the genetic background and disease risk and severity is often evaluated through traditional family-based linkage studies and positional cloning techniques. However, it is becoming increasingly clear that case-control studies based on association of disease or disease subphenotypes with candidate genes may have certain advantages over family pedigree studies for complex disease phenotypes. This is based in part on continued development of quantitative analysis and the discovery and availability of simple sequence repeats (SSRs) and single-nucleotide polymorphisms (SNPs). Linkage analyses with genetically standardized animal models also provide a useful tool to identify genetic determinants of host responses to environmental pollutants. Comparative mapping between the human and mouse genomes will yield candidate susceptibility genes that may be tested by association studies in human subjects. The combined human studies and mouse modeling likely will provide important insight to understanding genetic factors that contribute to differential susceptibility to pollutants in human populations. Support: HL57142, R826724, ES09606, and P30 ES00002

1854 ANIMAL STUDIES IN GENETIC SUSCEPTIBILITY.

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The contribution of genetic background to a biological process may be more easily determined and partitioned from environmental influence in the inbred mouse than in more heterogeneous species such as humans. The controlled standardized inbreeding of mice has led to genetic homogeneity that cannot be found or duplicated in human populations, making the inbred mouse an important source for genetic modeling of human diseases. Linkage relationships of homologous loci from human and mouse indicate that highly significant homology in gene order and chromosomal structure have been maintained since the evolutionary divergence of the human and mouse. Therefore, identification of the chromosomal location of a susceptibility gene in the mouse provides the basis for potentially localizing a homologous gene in the human. Recently, web-based tools have greatly enhanced searches for inter-strain homology (http://www.ncbi.nlm.nih.gov/HomoloGene/). The considerable resources and energy applied to the mapping of the mouse genome as an integral component of the Human Genome Project emphasize the importance of this murine model for human diseases. It is anticipated that, because the numbers of genes mapped in the human and mouse genomes are increasing almost exponentially with the advent of modern molecular genetic techniques, there will be similar increases in homologous association of disease genes in the two species.

1855 FROM MOUSE TO MAN: ACCELERATING DISCOVERY OF GENETIC SUSCEPTIBILITY LOCI FOR HUMAN DISEASE.

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The integrated use of a novel SNP-based genotyping method and gene expression analysis using high-density oligonucleotide microarrays has markedly increased the rate at which complex biological processes can be analyzed. Application of these technologies to murine experimental models of human inflammatory disease has enabled genetic susceptibility loci to be rapidly identified. As one example, a genetic locus regulating susceptibility to experimental allergic asthma in an experimental murine genetic model was identified using this approach. The identified murine susceptibility genes provide insight into pathways regulating human inflammatory disease susceptibility. We have also developed a SNP database and software that enable chromosomal regions regulating complex traits in mice to be computationally predicted. Several examples as to how the computational prediction program has been used to identify chromosomal regions regulating complex traits in mice will be presented. The correlation of computationally predicted regions and experimentally verified regions will be assessed.

1856 USING THE HUMAN GENOME PROJECT TO UNDERSTAND HUMAN VARIABILITY IN RESPONSE TO POLLUTANTS.

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Some human diseases are predominantly monogenic, but most diseases—including lung toxicity or cancer are polygenic (the combinatorial result of two, and usually more than a dozen or hundred, genes) and multifactorial (affected by epistasis and environmental factors). A polymorphism denotes the presence of two or more subgroups in any species population. Ford in the 1940s and Harris in 1980 stated that a polymorphism exists when the "commonest identifiable allele has a frequency of $\leq$=0.99 .." Polymorphic and rare variants have frequencies of $\geq$=0.01 and <0.01, respectively. Considering Hardy-Weinberg distribution ($p + q = 1.0$; $p^2 + 2pq + q^2 = 1$), q = the sum of the variant allele frequencies. If q = 0.01, the percentage of individuals homozygous for an autosomal recessive trait would be 0.0001% (1 in 10,000). All genes are polymorphic to varying degrees. Single-nucleotide polymorphism (SNP) consortia have compiled a map of 1-2 million SNPs, 85% of which are located within 5 kb of the nearest exon, but only 24 individuals of several ethnic backgrounds have been examined so far. In just the most recent generation of humans on this planet, every site at which mutations are compatible with life has been mutated an average of 240 times. Similar rates of mutation have occurred in every previous Homo generation. Classic neutral theory of population genetics allows us to infer from this rate that, by analyzing 99% of the most ethnically diverse 192 haplotype genomes on this planet, we will asymptotically approach 11 million ($q \geq 0.01$) SNPs; the number of rare SNPs will of course be much greater.On average, about 4-10 coding SNPs exist per gene, and 40-60% of these are expected to alter the gene's function. This computes to ~9.5 million SNPs (85% of 11 million) in the 5' and 3' flanks plus introns of the ~31, 000 genes, plus all cSNPs, which will make up 40-80 haplotypes per gene—with wide variation on a gene-by-gene basis. Ultimately, our knowledge of haplotype patterns of probably several hundred genes will be able to predict one's risk of lung toxicity or cancer caused by a particular inhaled pollutant.

1857 MITOCHONDRIAL TOXICITY PRODUCED BY ANTIVIRALS; MODELS AND MECHANISMS.

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The need for new treatments for serious viral infections, especially with HIV(Human Immunodeficiency Virus), HBV(Hepatitis B Virus), and HCV(Hepatitis C Virus), is urgent. Unfortunately, mitochondrial toxicity has impaired the development of nucleoside analogs, easily the most successful approach to treatment to date. The intracellular location of mitochondria, heritable defects, unique method of replication and susceptibility to effects on both electron transport and the oxidation of fatty acids combine to provide toxicologists with a formidable challenge. Pathophysiological implications of mitochondrial function include DNA that is unique to mitochondria (mtDNA), maternal inheritance of this special DNA, and regulation of intramitochondrial Ca^{2+} ions critical for normal function. Importantly, mitochondria control both the life and death of cells. The biogenesis of mitochondria is particularly dependent upon γ polymerase. Therefore chemicals, in particular nucleoside analogs, that inhibit or are a substrate for this enzyme can produce mitochondrial toxicity. This Symposium will link topics given above with the best understood but unfortunately fatal toxicity produced by the D

enantiomer of a pyrimidine nucleoside known as fialuridine. *in vitro* and *in vivo* experiments will compare and contrast effects on mitochondria produced by nucleside analogs and correlate these data with toxicity. Finally, research to develop novel molecular toxicological assays, including quantitative real-time PCR, will show how these techniques, coupled with data on uptake and phosphorylation of nucleoside analogs, can be used to predict the potential for mitochondrial toxicity. In addition to furthering our understanding of this enigmatic toxicity, this research will improve risk-benefit assessments and help to decrease this major impairment to the development of new antivirals.

1858 PATHOPHYSIOLOGICAL IMPLICATIONS OF MITOCHONDRIAL FUNCTION.

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Compared to other organelles of the cell mitochondria are unique in that they contain their own DNA. mtDNA encodes genes for several subunits of complexes I, III, IV, and V and genes for ribosomal proteins and tRNA. mtDNA is maternally inherited so that new sequence variants are transmitted along maternal lineages without the benefit of recombination with male mtDNA. Mutations in mtDNA often result in deficiencies in the respiratory chain causing defects in heart, skeletal muscle, and nervous system function. Toxic chemicals have long been known to disrupt mitochondrial metabolism and lead to cellular injury, but the mechanisms causing the injury are turning out to be more complex than expected. Mitochondria are normally a source of reactive oxygen species generation but enhanced generation of free radicals ultimately results in mitochondrial dysfunction. This can be due to direct damage to the mitochondrial respiratory components or enhanced inner membrane permeabilization, an event occurring during the mitochondrial permeability transition (MPT). The MPT initiates mitochondrial swelling, which ruptures the mitochondrial outer membrane, causes release of cytochrome *c* into the cytosol from the mitochondrial intermembrane space, and signals apoptosis. Mitochondrial ion regulation, such as free Ca^{2+}, is tightly regulated by Ca^{2+} uptake and release mechanisms across the membranes. Mitochondrial free Ca^{2+} levels may fluctuate to regulate mitochondrial ATP generation. Several pathophysiological conditions, however, can disrupt mitochondrial Ca^{2+} homeostasis, resulting in mitochondrial Ca^{2+} loading. Excess mitochondrial Ca^{2+} dissipates the membrane potential and can precipitate the MPT, causing bioenergetic failure and ultimately cell death. Thus, mitochondria are critical organelles both in the life and death of cells.

1859 INHIBITION OR PERTURBATION OF MITOCHONDRIA DNA SYNTHESIS BY ANTIVIRAL β-D-DIDEOXY-NUCLEOSIDE ANALOGS COULD BE ONE OF THE KEY MECHANISMS RESPONSIBLE FOR THEIR TOXICITY.

Y. Cheng. *Yale University, New Haven, CT.* Sponsor: G. Szczech.

β-D-dideoxy-nucleoside analogs are class of compounds having anti-cancer and anti-viral activity. The major limiting toxicity encountered is manifested in proliferating tissues. Several β-D-dideoxy-nucleoside analogs were found to have anti-HIV activity and were used on a long-term basis for the treatment of patients with AIDS. At a clinically relevant dose, those compounds exerted toxicity at non-proliferating tissues after a period of time of usage. One of the mechanisms responsible for their delayed toxicity in non-proliferating tissue is that depletion of mitochondrial DNA (mt-DNA) no longer supports mt function in tissues that were toxified. Those compounds exert their action on mt-DNA through DNA polymerase γ by terminating mt-DNA elongation, which is needed for mt-DNA replication or repair. However, the delayed toxicity is reversible due to mt-exonuclease by removing the analogs from the mt-DNA terminal once the treatment is terminated. In contrast, the delayed toxicity of nucleoside analogs such as FIAC or FMAU, which could be internalized into mt-DNA through the action of DNA polymerase γ and affect mt-DNA synthesis as well as transcription, is irreversible since mt does not have the mechanism to remove the internal incorporated nucleoside analog. For the treatment of viral diseases, an ideal nucleoside analog only acts on viral DNA synthesis apparatus but not nuclear or mt-DNA synthesis of uninfected cells. In the process of looking for such analogs, L(-)SddC was discovered to have potent anti-HBV and HIV activity by several laboratories including mine but does not have potent activity as other anti-HIV β-D-dideoxy-nucleoside analogs against mt-DNA synthesis. The lack of action is due to the lack of uptake of the active metabolite in mt although the DNA polymerase γ could still utilize L(-)SddC-TP as a substrate resulting in DNA chain termination. A summary of our work and understanding in terms of the mechanism of those anti-HIV and anti-HBV nucleoside analogs in affecting mt-DNA synthesis will be presented.

1860 MITOCHONDRIAL TOXICITY OF FIAU AND OTHER ANTIVIRAL NUCLEOSIDE ANALOGS: MECHANISMS AND METHODS OF EVALUATION.

J. M. Colacino. *Infectious Diseases Research, Lilly Research Labs, Indianapolis, IN.* Sponsor: G. Szczech.

FIAU is a thymidine nucleoside analog (NA) with antiviral activity against hepatitis B virus (HBV) *in vitro* and in animal models of infection. Initially, FIAU demonstrated clinical promise for the treatment of chronic HBV infection but in further clinical trials, long term administration of FIAU resulted in fatal multiorgan toxicity with a delayed onset characterized by refractory lactic acidosis. The clinical manifestations were consistent with mitochondrial dysfunction. FIAU was demonstrated to have effects on mitochondrial function in a number of experiments including evaluation of mitochondrial (mt) DNA replication and production of lactic acid in cells treated with FIAU; measurement of incorporation of FIAU into mtDNA; interaction of FIAU-TP with cellular DNA polymerase gamma which replicates mtDNA; and EM examination of mitochondrial morphology in cells treated with FIAU. FIAU was orally administered to woodchucks uninfected or chronically infected with woodchuck hepatitis virus in an effort to simulate the clinical trials. Whereas no adverse events were observed in animals that received clinically relevant doses of FIAU for 28 days, animals that received FIAU for 12 weeks died or were euthanized 78 to 111 days after initiation of treatment. Among other toxicities, these animals demonstrated hepatic insufficiency, lactic acidosis, and hepatic steatosis in the final stages of FIAU toxicity. These findings were similar to those seen in humans treated orally with FIAU and are consistent with a deleterious effect on mitochondrial function. Some NAs that are used for the treatment of AIDS can cause a delayed toxicity the symptoms of which include myopathy, peripheral neuropathy, deafness, pancreatitis, hepatocellular failure, and lactic acidosis. These symptoms are consistent with mitochondrial dysfunction and might be mediated through interactions of the NA-triphosphate with DNA polymerase gamma. The experience with FIAU underscores the need for a systematic evaluation of the effects of antiviral nucleoside analogs on mitochondrial function.

1861 MECHANISMS OF ANTIVIRAL NUCLEOSIDE ANALOGUE-INDUCED MITOCHONDRIAL TOXICITY.

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Chronic treatment with nucleoside analogues (NRTIs) used in treatment of HIV and HBV has been linked to lactic acidosis, hepatotoxicity, myopathy, pancreatitis, peripheral neuropathy and lipodystrophy. A common mechanism of mitochondrial toxicity has been proposed. Mitochondria each contain 2 - 3 DNA strands (mtDNA) with thirteen genes encoding enzymes in the electron transport chain. mtDNA replication is independent of nuclear replication but requires DNA polymerase γ (DNA pol γ), an enzyme encoded by nuclear DNA. DNA pol γ binds and inserts endogenous nucleoside triphosphates into the growing DNA strand, but also binds many NRTIs. Most NRTIs are chain terminators of mtDNA replication, but some (e.g. fialuridine) are incorporated into mtDNA. Impaired cell function or cell death follows the gradual depletion of mtDNA to between 15 and 20% of normal cell content. In some cases there is a correlation between inhibition of DNA pol γ and toxicity *in vivo*. Zalcitabine (ddC), for example, is the most potent inhibitor of DNA pol γ and also the most potent inducer of peripheral neuropathy. This correlation does not always hold. Both ddC and d4T (stavudine) are more potent inhibitors of DNA pol γ than AZT (zidovudine), but only AZT has been associated with clinical signs of myopathy, and evidence of mitochondrial damage in muscle cells in experimental studies in rodents. In our own laboratory, we have shown that NRTI inhibition of adipogenesis precedes depletion of adipocyte mtDNA *in vitro*, indicating that mitochondrial toxicity may not explain lipodystrophy associated with highly active antiretroviral therapy (HAART) for HIV. This presentation will review the evidence for mitochondrial toxicity as an explanatory mechanism for all adverse side effects of NRTI treatment.

1862 NOVEL MOLECULAR TOXICOLOGICAL ASSAYS FOR ANTIVIRAL AGENTS.

L. Stuyver. *Pharmasset, Tucker, GA.* Sponsor: G. Szezech.

One of the most preferred targets for the development of the drugs viral infections has been the nucleic acid synthetic machinery. Nucleoside analogs are phosphorylated intracellularly to their triphosphates and can either incorporate into viral nucleic acids or inhibit the viral polymerases. For a compound to be considered selec-

tive and potentially useful for drug development, it must preferentially target viral polymerases and minimally affect cellular polymerases. The search for potent and non-toxic compounds requires expertise in sophisticated toxicological assays that can reduce the risk of potential problems in humans treated chronically with antiviral agents at relevant and effective concentrations. Stereospecific nucleosides that target the polymerase are versatile molecules with well-established biochemical activation mechanisms and pharmacology. They continue to provide a rich source of anti-HIV and HBV agents, although other classes of compounds are beginning to emerge. Some of these new molecules have the desired selectivity and potency *in vitro* and in animal models, as well as a unique resistance profile. Our laboratory has used cellular assays as well as enzyme assays to determine the effect of nucleosides on mitochondrial function and morphology. Using quantitative real-time PCR (Q-RT-PCR) coupled with cellular drug uptake and phosphorylation assays, the relative toxicity of new nucleoside analogs can be determined reproducibly and rapidly. These novel molecular toxicological assays will further aid in designing improved treatment protocols. The attendees of this symposium will gain a better understanding of the opportunities and challenges in anti-HIV and HBV drug discovery, and the systematic approach for developing new safe compounds for Phase 1 clinical trials.

1863 SAFETY ASSESSMENT OF FOODS DERIVED FROM BIOTECH FOOD CROPS - INTRODUCTORY REMARKS.

G. A. Burdock. *Burdock Group, Vero Beach, FL.*

The current "generation" of toxicologists is faced with the challenge of ensuring the safety of biotechnologically produced foods and food ingredients. Food holds a position of inviolability in our culture because of the involuntary nature of its consumption and the pleasure with which fulfill our nutritional needs. This cultural demand for the absolute purity of food is reflected in literal interpretations of the Delaney clause with no room for *de minimus* considerations. This demand for absolute purity is under assault with seeds of doubt about the integrity and thoroughness of the vetting process for safety of biotechnologically produced substances and has given rise to generalized fears, as exemplified by the Precautionary Principle. This workshop examines key concepts for safety evaluation of biotechnologically produced foods and includes a look at some critical lessons gained from safety evaluation of irradiated food, a strikingly similar problem. What is accomplished within the next 24 months, in terms of safety evaluation of biotechnologically produced food, will profoundly affect the nature and abundance of the food supply for the next three generations of the world's population.

1864 COMPLEXITY OF TOXICOLOGICAL STUDIES OF IRRADIATED FOODS.

A. Brynjolfsson. *Applied Radiation Industries, Wayland, MA.* Sponsor: G. Burdock.

The Surgeon General of the US Army initiated preliminary studies on safety of some irradiated foods in 1948, and short-term studies of 54 foods in 1954, followed by long-term multi-generation animal feeding studies of 21 foods. December 1953 President Eisenhower in an address before the United Nations urged international cooperation and acceptance of the "Atoms for Peace Program". This led to the first International Conference on Peaceful Uses of Atomic Energy. Many countries became then involved in studies of irradiated foods' wholesomeness. Finally, in the 80's and 90's irradiated foods became generally recognized as wholesome. Food is important in international trade and its safety requires worldwide acceptance, open scientific cooperation and exchange of information across the globe. Not only the Health Authorities of each country must accept the results, but also United Nations' WHO and FAO, as well as the Codex Alimentarius, and WTO. We must assure ourselves scientifically that no harmful effects could go undetected, and we must also reassure the public. The word irradiation leads to many misconceptions and dispelling suspicion of harmful effect becomes very difficult. When the evaluation of the data shows that the test group is doing better than the control, the test-group is considered normal or showing no detrimental effect. However, if the control group does better than the test-group, the test-group is suspect. Multi-generation studies can multiply the probability for the test-group to become suspect. These variations may be completely unrelated to the diets. Detailed qualitative and quantitative analyses of all the chemical changes in foods when irradiated, and realization that these changes are similar to those normally occurring in foods when stored and processed by conventional methods, as well as feeding concentrates of the radiolytic products to animals in smaller studies were important in proving beyond reasonable doubt that irradiated foods are safe and wholesome. In all this, openness and international scientific cooperation is important as food and food processing requires international acceptance.

1865 OECD/FAO SUBSTANTIAL EQUIVALENCE FRAMEWORK FOR WHOLE FOOD SAFETY ASSESSMENT.

I. C. Munro. *CANTOX Health Sciences International, Mississauga, ON, Canada.*

OECD/FAO has developed the concept of "substantial equivalence" to assess the safety of foods derived from biotech food crops. This concept involves the comparison of key nutrient/antinutrient composition between biotech crop varieties and existing varieties from which they were derived. Many thousands of compositional comparisons are made for crops grown across multiple geographies and over 2 or more growing seasons. Extensive data bases are being developed to establish the normal range of composition in existing commercial crop varieties. If the composition of food derived from the biotech crops is "substantially equivalent" to existing varieties, then the safety assessment focuses on the defined difference between the new and existing varieties, e.g. the trait(s) introduced by genetic engineering (introduced protein). Several biotech crops, such as herbicide resistant corn, canola, soybeans, and cotton, as well as insect protected corn and cotton, have undergone this assessment and have been shown to be substantially equivalent to commercial crop varieties. If existing varieties of a crop are being improved to increase nutrient levels (e.g. increased levels of essential amino acids, or fatty acids) through genetic engineering, then the new crop variety is by definition "non - substantially equivalent" to existing crop varieties. Strategies for assessing the safety of nutritionally improved "non-substantially equivalent" crop varieties are similar to those described above and would include a nutritional/safety assessment of intended changes in composition of the crop.

1866 SAFETY ASSESSMENT OF PROTEINS INTRODUCED INTO FOOD CROPS.

S. L. Taylor. *Food Science & Technology, University of Nebraska, Lincoln, NE.*

The safety assessment of protein products of genes introduced into food crops is multifaceted. The most important food safety concern is to assess the allergenic potential of the introduced protein. This includes comparing the amino acid sequence of the introduced protein to data bases of known protein food allergens to confirm that the protein is not related to known allergens. The levels of accumulation of the introduced protein in food parts of the plant and assessment of its potential digestibility are key learnings. Known protein allergens tend to be present at abundant levels in the food and are generally resistant to digestion by proteases in the GI tract. The mode of action of the introduced protein should be known, e.g. if it is an enzyme, what are its substrates and the catalytic products. The amino acid sequence of the introduced protein needs to be compared to data bases for known toxic/antinutrient proteins to confirm the absence of similarity of the introduced protein to known toxicants. Since known protein toxicants often act through acute mechanisms to cause toxicity when ingested, the potential acute toxicity of the introduced protein can be tested by administration by gavage at high dosages to rodents.

1867 ROLE OF COMPOSITIONAL ANALYSES AND SUB-CHRONIC TOXICITY STUDIES IN THE EVALUATION OF SUBSTANTIAL EQUIVALENCE OF BIOTECH FOOD CROPS.

W. P. Ridley, B. G. Hammond and J. D. Astwood. *Product Safety Center, Monsanto Company, St. Louis, MO.*

Extensive compositional analyses including nutrients, anti-nutrients and secondary metabolites are conducted to evaluate the nutritional status of food crops produced through biotechnology. The results of these compositional evaluations are compared with the results obtained for parental control varieties and conventional commercial varieties in order to examine the placement of the biotech food crop within the natural variability seen for an individual biochemical component. This extensive biochemical sampling, although directed towards nutritional parameters, also assesses unexpected effects due to either the expression or the insertion of the introduced genes. To further assess the potential for an unintended or unexpected effect, food crops produced through biotechnology may be incorporated into the diets for sub-chronic toxicity studies in rats. Various toxicological endpoints are evaluated in rats including body weight gain, hematology, clinical chemistry, urine chemistry, organ weights at necropsy and, gross and microscopic pathology. The food and feed safety assessment of Roundup Ready® corn (GA21) included both compositional and toxicological studies. Compositional analysis results showed that, except for a few minor differences that are unlikely to be of biological significance, the grain and forage of GA21 corn were comparable to that of the control corn line and to conventional corn (Sidhu et al, *J. Agricultural Food Chem*, **2000**, 48, 2305-2312). Similar results were obtained for the toxicological endpoints - the magnitude of the significant differences was small and the values were within historical limits. These data taken together demonstrate that Roundup Ready® corn is as safe and nutritious as conventional corn for food and feed use.

1868 THE USE OF ANIMAL FEEDING STUDIES TO CONFIRM THE SAFETY/WHOLESOMENESS OF NEW CROP VARIETIES.

B. Glen. *Federation of Animal Science Societies, Bethesda, MD.* Sponsor: G. Burdock.

The US regulatory review process for new crop varieties is accomplished jointly by three federal agencies through a science-based, integrated and multi-disciplinary approach. To date, more than 50 crops produced through biotechnology have been approved for commercialization and these have been substantially equivalent to conventional counterparts for nutrient content and known toxicants. For crops approved to date, feeding studies using livestock have not been required as part of the regulatory process. Further, the US Food and Drug Administration considers the consumption of DNA to be "Generally Regarded as Safe." Nevertheless, to reassure the users of these crops that the food/feed is safe, numerous research studies have been undertaken in various farm animal species around the world. Many new crop varieties that are herbicide tolerant or insect protected (eg corn, soy, canola, cotton) have been fed to chickens, beef and dairy cattle, swine, sheep and fish in universities around the world using commercial feeding conditions. Findings from over 30 independently conducted studies have indicated no differences for nutrient composition, digestibilities, and animal performance when livestock were fed feedstuffs from conventional and biotech crops. Furthermore, in independent studies conducted to determine whether transgenic DNA and proteins can be detected in animal products, no transgenic plant-source DNA and proteins have been found in milk, meat, and eggs.

1869 TRANSGENIC MODELS IN REPRODUCTIVE TOXICOLOGY: APPLICATIONS IN MECHANISTIC AND APPLIED RESEARCH.

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The development of transgenic models has proven to be an invaluable tool for dissection of critical pathways in embryo-fetal development and reproduction. Phenotypic characterization of such models have helped to define critical roles for specific genes and molecules in pattern formation, differentiation and function. Only recently have such models been applied to toxicological investigations. This workshop explores the application of transgenic models in mechanistic and applied toxicological research. Comparison of phenotypes resulting from gene inactivation with patterns of toxicity can provide insight regarding the mechanisms through which agents/compounds act. The models can also be used to directly test hypotheses regarding the roles of specific genes and pathways in the toxic response by administering the test agent(s) to knockout animals. Although the workshop will focus on investigations in developmental and reproductive toxicology, these models also have value in drug development and evaluation of pharmacologic action in non-reproductive tissues/organs. Such an application will be discussed in which a transgenic model was used to evaluate and develop candidate compounds based on specific mechanistic responses. This workshop will present current applications of transgenic models in toxicology and stimulate discussions of future applications in both mechanistic and applied research.

1870 TRANSGENIC MOUSE MODELS FOR GENE - NUTRITION INTERACTIONS DURING EMBRYONIC DEVELOPMENT.

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Congenital abnormalities associated with maternal diabetes include neural tube defects, cardiovascular malformations, and caudal regression/sacral agenesis, and are referred to as diabetic embryopathy. We found such defects can be phenocopied in Isl-1-transgenic mice. Isl-1 is essential for differentiation of motor neurons in the spinal cord and proper pancreatic development. This functional spectrum is similar to that of the HLXB9 gene, which is linked to human sacral agenesis (Currarino Syndrome). The parallel developmental phenotypes of HLXB9 mutations in humans and Isl-1 misexpression in mice imply that transcription factors from one cellular pathway can cause comparable pathogenesis in the developing embryo. These phenotypes are not a general property of homeobox transcription factors: classical Hox genes induce different phenotypes that may be modulated by nutrition. We have initiated a genome-wide search for genes regulated by Isl-1 to understand the mechanistic aspects how de-regulated Isl-1 expression can cause developmental defects. Using transgenic mice as tools for gene discovery and gene classification, we have identified target genes on mouse chromosome 9. We have now started to analyze regulatory elements in this gene cluster *in vivo*. Nature and location of these control elements suggest that Isl-1 is one part of a complex regulatory pattern. We submit that de-regulation of Isl-1 expression could be responsible for congenital defects found in human diabetic embryopathy, suggesting that metabolic factors contribute to the control of Isl-1 expression. To test this hypothesis, we have developed a method to screen large genomic regions for the presence of regulatory elements *in vitro* and *in vivo*. The identification of such elements will provide information on upstream factors that regulate Isl-1 expression and the influence of metabolic status on gene expression in diabetic embryopathy.

1871 EPIDERMAL GROWTH FACTOR RECEPTOR PATHWAY IN DEVELOPMENTAL TOXICITY: KNOCKOUT MODELS IN MECHANISTIC TOXICOLOGY RESEARCH.

B. D. Abbott and A. R. Buckalew. *Reproductive Toxicology Division, USEPA, Research Triangle Park, NC.*

Epidermal growth factor (EGF) and related factors regulate proliferation and differentiation of cells in many tissues and represent important signal transduction pathways in embryo/fetal development. The EGF receptor (EGFR) has six ligands (including EGF and transforming growth factor-alpha, TGF-alpha) with tissue-specific expression patterns. This pathway appears to be involved in the induction of cleft palate and hydronephrosis following exposure to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD). TCDD exposure *in utero* alters proliferation of palatal and urinary tract epithelial cells and is associated with disrupted expression of EGFR, EGF and/or TGF-alpha. Transgenic knockout (KO) mice which do not express EGF, TGF-alpha or either growth factor (EGF+TGF) offer an interesting model for inquiry regarding the role of these peptides in mediating toxicity. Teratogenic responses in EGF KO, TGF KO, and EGF+TGF KO mice were evaluated following gestational exposure to TCDD. The tissue-specific dose-response profiles indicated that expression of EGF, TGF-alpha or both influenced the incidence and severity of teratogenic responses. For cleft palate, the absence of EGF muted the response, suggesting an EGF-mediated effect. In the urinary tract, the absence of EGF increased responsiveness and this may be related to effects of TCDD on the expression of TGF-alpha in ureteric epithelial cells. These transgenic models provide a means to discriminate the sensitivity of target tissues based on the role of specific ligand(s) in mediating the toxic response. Thus, transgenic models can help evaluate multiple components of complex, interactive pathways, such as those for signal transduction, and can provide important insight into mechanisms of toxicity. This abstract does not necessarily reflect EPA policy.

1872 HYPOTHESIS TESTING IN MALE REPRODUCTIVE TOXICOLOGY USING MUTANT MICE.

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Several different mutant mouse models have been used to test mechanisms by which male reproductive toxicants trigger germ cell apoptosis. The Fas system is a paracrine pro-apoptotic signaling pathway; in the testis, Fas ligand (FasL) is expressed by Sertoli cells and induces apoptosis of germ cells expressing Fas receptor. p53 is a transcription factor which modulates the expression of bcl-2 family members, Fas receptor, and other elements of the apoptotic machinery. To examine the possible interactions between the Fas system and p53, germ cell apoptosis was measured in deficient mice exposed to a germ cell toxicant (radiation) or a Sertoli cell toxicant (mono-(2-ethylhexyl)phthalate, MEHP). Double deficient mice lacking both FasL and p53 and p53 knockout mice were highly resistant to radiation-induced germ cell apoptosis, while FasL deficient mice were as sensitive as wild type mice to this injury. In contrast, upon exposure to MEHP, all of the deficient strains were protected compared to wild type. These results indicate that the apoptotic response to radiation is p53-dependent and FasL-independent while the apoptotic response to MEHP is at least partially dependent on both FasL and p53. A second set of experiments evaluated testicular injury in transgenic mice expressing both a normal endogenous ubiquitin and a dominant negative mutant human ubiquitin that prevents poly-ubiquitination and thus frustrates the processing of abnormal and short-lived proteins involved in signal transduction. When these transgenic mice were made cryptorchid, their testes were more sensitive to this heat-induced injury than wild type controls. These results identify the processing of abnormal and short-lived proteins as an important mechanism underlying the response to injury in the testis, and suggest that impaired activation of polyubiquitination-dependent

signal transduction pathways, such as the anti-apoptotic transcription factor NF-kB, could play a role in the increased sensitivity of the mutant ubiquitin mouse testis.

1873 USE OF GENE KNOCKOUTS TO ESTABLISH A MOLECULAR FRAMEWORK FOR TOXICANT-INDUCED OOCYTE APOPTOSIS IN MURINE AND HUMAN OVARIES.

J. L. Tilly. *Vincent Center for Reproductive Biology, Department of OB/GYN, Massachusetts General Hospital/Harvard Medical School, Boston, MA.* Sponsor: D. Shuey.

For many years my Lab. has studied the role of programmed cell death (PCD) or apoptosis in normal and pathological depletion of germ cells from the ovaries (Dev Biol 1999 217:1-13). A powerful approach to understanding how chemicals kill oocytes has been to examine the impact of "knocking out" PCD regulatory genes, including those encoding signaling molecules (acid sphingomyelinase or ASMase), integrators (Bcl-2 family members) and executioners (caspases), on toxicant-induced oocyte apoptosis in mice (Mol Endocrinol 2001 15:845-853). The evolutionary conservation of the findings is then tested by exposing human ovarian tissue grafted in immunodeficient mice to the same insult and evaluating human oocyte responses. Two examples of our work with gene knockouts in female reprotoxicology will be discussed. The first example describes our efforts to elucidate the intracellular mechanisms by which the widely-used anticancer drug, doxorubicin, activates oocyte apoptosis. These studies have shown that ASMase-generated ceramide, the pro-apoptotic Bcl-2 family member Bax, and the PCD executioner enzyme caspase-2 are needed for oocytes to die in response to this toxicant (Nature Med. 1997 3:1228-1232; Genes Dev 1998 12:1304-1314; Nature Med. 2000 6:1109-1114). The second example concerns the identification of PCD-related genomic targets for the aromatic hydrocarbon receptor (AHR) in oocytes that are required for the ovotoxic effects of polycyclic aromatic hydrocarbons (PAHs). A novel intracellular signaling pathway involving AHR-driven transcription of the gene encoding Bax in oocytes will be discussed in detail, along with results from parallel studies of human oocyte responses to PAH using a human ovarian xenograft model (Nature Genet 2001 28:in press). Collectively, these studies using gene knockout technologies have identified functionally important biochemical and genetic pathways in oocytes that, when perturbed by xenobiotics, trigger female germ cell death *via* apoptosis (supported by NIH R01-ES08430).

1874 THE USE OF KNOCKOUT MICE TO DISSOCIATE INDUCTION OF THE HEPATIC MICROSOMAL MIXED-FUNCTION OXIDASE (MFO) SYSTEM FROM INHIBITION OF THE 5-LIPOXYGENASE (5-LO) ENZYME.

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Leukotrienes (LTs) are biologically active metabolites of arachidonic acid (AA) that function in normal and pathophysiologic conditions. The initial step in LT biosynthesis is the ezymatic conversion of AA to LTA_4 by 5-LO. Initially developed to understand the role of LTs in inflammatory conditions, we tested 5-LO -/- mice in the collagen-induced arthritis assay. Since this assay is most effectively established in DBA/1 inbred mice, we developed a congenic strain of 5-LO -/- mice for phenotypic analysis by consecutive backcrossing for 10 generations onto the DBA/1 genetic background. This effort demonstrated that 5-LO deficient mice had reduced inflammatory responses in the collagen-induced arthritis assay. Since LTs have also been implicated in asthma, a variety of anti-LT therapies have been tested for efficacy in treating the disease. We found in preclinical testing that the 5-LO inhibitors zileuton (Abbott), CJ-11, 802 (Pfizer) and ZD2138 (Zeneca) all induce the hepatic microsomal MFO system of rodents. Since these three agents are structurally dissimilar, we hypothesized that MFO induction by 5-LO inhibitors could be pharmacologically-mediated. To test this hypothesis, male wild-type and 5-LO -/- mice were administered either CJ-11, 802 (200 mg/kg/day) or vehicle by oral gavage for 14 consecutive days. At necropsy, various indices of MFO induction (e.g. liver weight, cytochrome P450 content, and cytochrome c reductase) were measured and all were increased similarly in the treated wild-type and 5-LO -/- mice compared to corresponding controls. These results indicate that induction of the hepatic microsomal MFO system in rodents by 5-LO inhibitors is not related to altered LT status.

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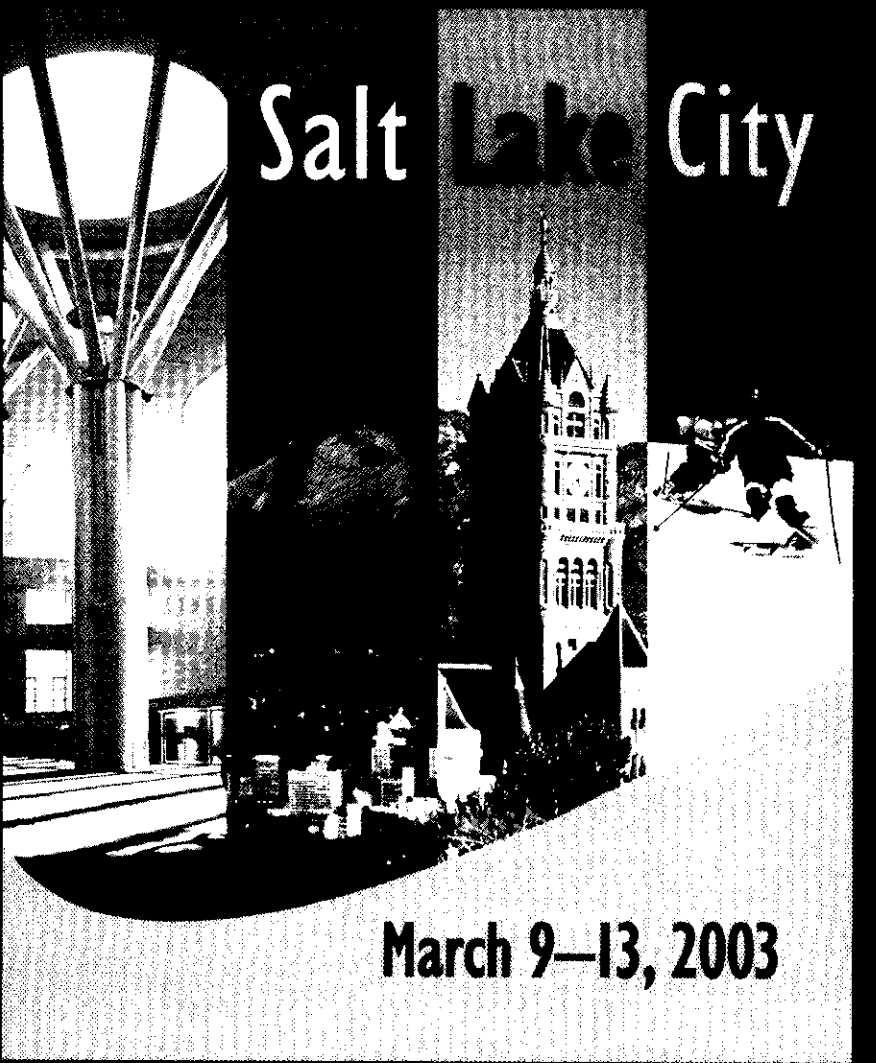

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